PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07D 207/16, A61K 31/40
C07D 405/12, 401/12

A1

(11) International Publication Number: **WO 92/14706**

(43) International Publication Date: 3 September 1992 (03.09.92)

(21) International Application Number: PCT/EP92/00321

(22) International Filing Date: 12 February 1992 (12.02.92)

(30) Priority data:
9103454.6 19 February 1991 (19.02.91) GB

(71) Applicant *(for GB only):* PFIZER LIMITED [GB/GB]; Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(71) Applicant *(for all designated States except GB US):* PFIZER INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

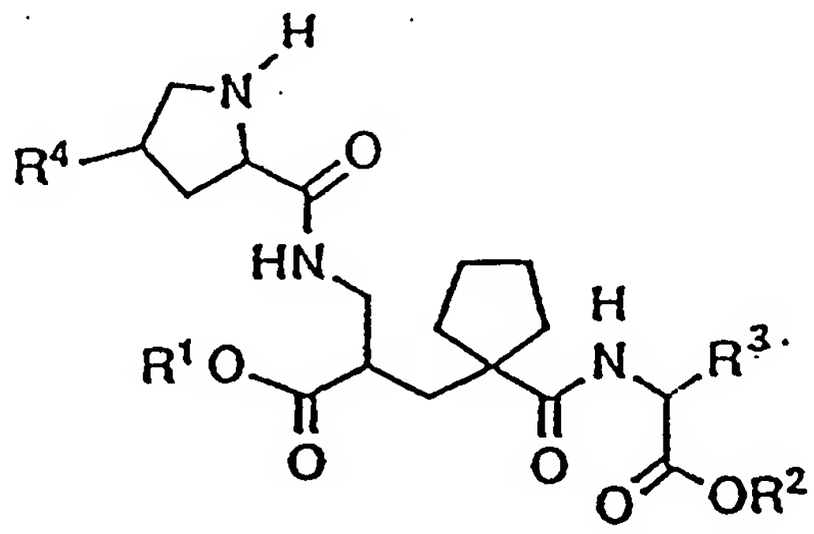
(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* BROWN, David [GB/GB]; Pfizer Central Research, Ramgsgate Road, Sandwich, Kent CT13 9NJ (GB). COLLIS, Alan, John [GB/GB]; DANILEWICZ, John, Christopher [GB/GB]; JAMES, Keith [GB/GB]; KOBYLECKI, Ryszard, Jurek [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(74) Agents: MOORE, James, William et al.; Pfizer Limited, Patents Department, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

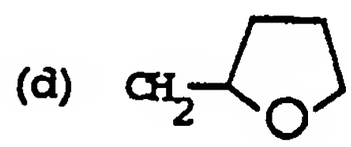
(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

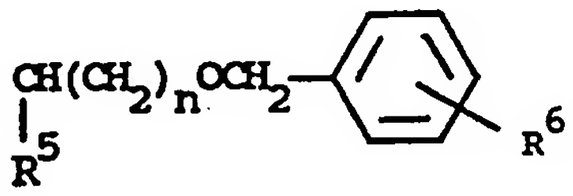
(54) Title: CYCLOPENTANE-DERIVED GLUTARAMIDE ANTIHYPERTENSIVE AGENTS



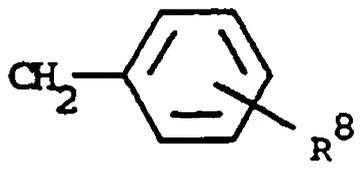
(I)



(a)



(c)



(57) Abstract

Compounds of formula (I), wherein R^1 and R^2 are each independently H or a biolabile ester-forming group, and either or both of OR^1 and OR^2 may optionally be replaced by NH_2; R^3 is (a), wherein R^5 is H or methyl, R^6 is H or halo, and n is O or 1; (b) CH_2OR^7, wherein R^7 is C_1-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_7 cycloalkyl, (C_1-C_4 alkoxy)C_1-C_6 alkyl, (C_1-C_4 alkoxy)C_3-C_6 alkenyl, (halo)C_3-C_6 alkenyl, (C_3-C_7 cycloalkyl)C_1-C_6 alkyl or (CF_3)C_1-C_6 alkyl; (c) wherein, R^8 is CH_2OH, CH_2OCH_3, $OCH(R^5)CH_2OH$ or $OCH_2CH_2OCH_3$ and R^5 is a previously defined; (d) or (e) (C_1-C_4 alkoxy)C_3-C_6 alkenyl or (C_1-C_4 alkoxy)-C_2-C_6 alkyl; and R^4 is H or hydroxy; and pharmaceutically acceptable salts thereof, are diuretic and natriuretic agents having utility in the treatment of hypertension, heart failure, renal insufficiency and other disorders.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FI**	Finland	**ML**	Mali
AU	Australia	**FR**	France	**MN**	Mongolia
BB	Barbados	**GA**	Gabon	**MR**	Mauritania
BE	Belgium	**GB**	United Kingdom	**MW**	Malawi
BF	Burkina Faso	**GN**	Guinea	**NL**	Netherlands
BG	Bulgaria	**GR**	Greece	**NO**	Norway
BJ	Benin	**HU**	Hungary	**PL**	Poland
BR	Brazil	**IE**	Ireland	**RO**	Romania
CA	Canada	**IT**	Italy	**RU**	Russian Federation
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU**	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		
ES	Spain	**MG**	Madagascar		

CYCLOPENTANE-DERIVED GLUTARAMIDE ANTIHYPERTENSIVE AGENTS

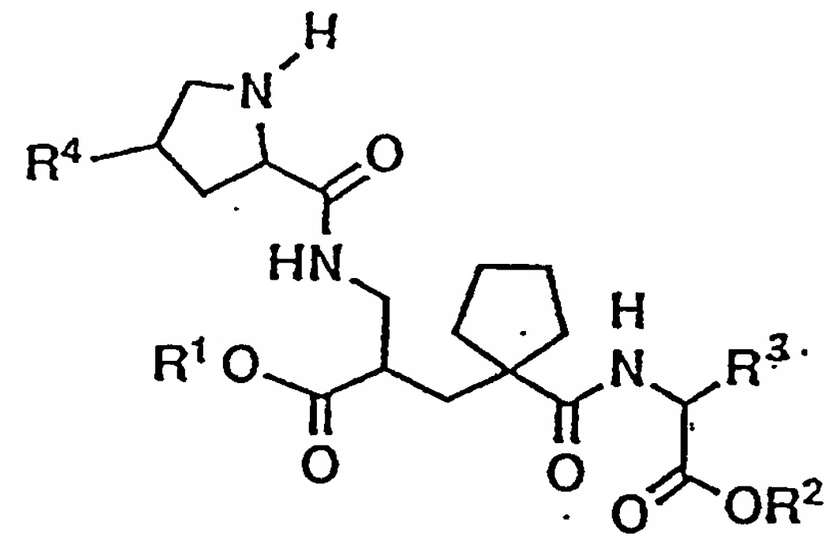
This invention relates to a series of cyclopentyl-substituted glutaramide derivatives which are antihypertensive agents having utility in the treatment of various cardiovascular disorders, including hypertension and heart failure.

According to the specification of our European patent application 0358398, we disclose certain cycloalkyl-substituted glutaramide derivatives which are inhibitors of the zinc dependent enzymes neutral metalloendopeptidase (E.C. 3.4.24.11) and angiotensin converting enzyme. Thus these compounds have a dual pharmacological action by inhibiting two key enzymes involved in blood pressure control, which makes them particularly useful in the treatment of various forms of hypertension and associated cardiovascular disorders, e.g. congestive heart failure and glaucoma.

The present invention includes further novel cyclopentyl-substituted glutaramide diacids which also possess said dual enzyme inhibitory activity. More specifically the invention provides biolabile (and thus prodrug) monoester, diester, monoamide, diamide and monoester-monoamide derivatives of the compounds, which have improved oral bioavailability profiles over those of the bioprecursors disclosed in EP-A-0358398. That is, after oral administration of the prodrugs disclosed herein, significantly enhanced systemic levels of the derived diacids are achieved. Without wishing to be bound by any particular mechanism of action, this is thought, at least in part, to be due to their improved resistance to breakdown by gastrointestinal enzymes, which allows the compounds to be more fully absorbed before

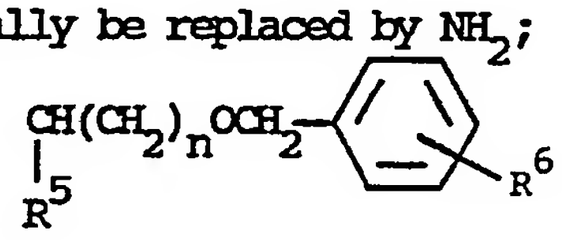
conversion to the active diacid species takes place.

The compounds are of the formula:



(I)

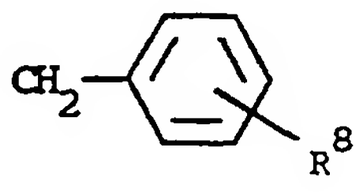
wherein R^1 and R^2 are each independently H or a biolabile ester-forming group, and either or both of OR^1 and OR^2 may optionally be replaced by NH_2;

R^3 is (a) 

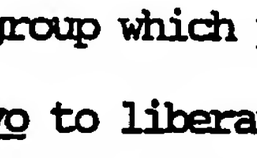
wherein R^5 is H or methyl, R^6 is H or halo, and n is 0 or 1;

(b) CH_2OR^7

wherein R^7 is C_1-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_7 cycloalkyl, (C_1-C_4 alkoxy)C_1-C_6 alkyl, (C_1-C_4 alkoxy)C_3-C_6 alkenyl, (halo)C_3-C_6 alkenyl, (C_3-C_7 cycloalkyl)C_1-C_6 alkyl or (CF_3)C_1-C_6 alkyl;

(c) 

wherein R^8 is CH_2OH, CH_2OCH_3, $OCH(R^5)CH_2OH$ or $OCH_2CH_2OCH_3$ and R^5 is as previously defined;

(d)  or

(e) (C_1-C_4 alkoxy)C_3-C_6 alkenyl or (C_1-C_4 alkoxy)C_2-C_6 alkyl;

and R^4 is H or hydroxy;

and include pharmaceutically acceptable salts thereof.

In the above definitions halo means fluoro, chloro, bromo or iodo. Alkyl groups having three or more carbon atoms, and alkenyl or alkynyl groups having four or more carbon atoms, may be straight or branched-chain.

The term biolabile ester-forming group is well understood in the art as meaning a group which provides an ester which can be readily cleaved in vivo to liberate the corresponding acid.

In the case of the compounds of formula (I), such biolabile mono- or diester prodrugs are particularly advantageous in providing compounds of the formula (I) suitable for oral administration. The suitability of any particular ester-forming group can be assessed by conventional in vivo animal or in vitro enzyme hydrolysis studies. Thus desirably, for optimum effect, the ester should only be hydrolysed after absorption is complete. Accordingly, the ester should be resistant to premature hydrolysis by digestive enzymes before absorption, but should be productively hydrolysed by, for example, gut-wall, plasma or liver enzymes. In this way, the active diacid is released into the bloodstream following oral absorption of the prodrug.

Suitable biolabile esters include alkyl, alkanoyloxyalkyl, cycloalkanoyloxyalkyl, aroyloxyalkyl and alkoxycarbonyloxyalkyl esters, including cycloalkyl and aryl substituted derivatives thereof, aryl esters, cycloalkyl esters, haloalkyl esters, oxoalkyl esters, dihydroxyalkyl esters including ketal derivatives thereof, pyridyl esters and [4-(5-alkyl or 5-aryl-1,3-dioxolen-2-onyl]methyl esters, wherein said alkanoyl or alkyl groups may contain from 1 to 8 carbon atoms and be branched or straight chain, said cycloalkyl groups may contain from 3-8 carbon atoms and said cycloalkanoyl groups from 4-8 carbon atoms wherein both are optionally benzo-fused, and said aryl groups are phenyl, naphthyl or indanyl optionally substituted with one or more C_1-C_4 alkyl, C_1-C_4 alkoxy or C_1-C_4 alkoxycarbonyl groups or with halo atoms.

Thus examples of R^1 and R^2 when they are biolabile ester-forming groups include C_1-C_5 alkyl, C_5-C_7 cycloalkyl, (cyclohexyl)C_1-C_3 alkyl, (phenyl)C_1-C_3 alkyl, 1-(C_2-C_5 alkanoyloxy)C_1-C_4 alkyl, 1-(C_5-C_6 cycloalkylacetoxy)C_1-C_4 alkyl, 1-(C_5-C_7 cycloalkylcarboxy)C_1-C_4 alkyl, 1-(2-indanylcarboxy)C_1-C_4 alkyl, 1-(benzoyloxy)C_1-C_4 alkyl, 3-phthalidyl, 1-(C_1-C_4 alkoxy-carbonyloxy)C_1-C_4 alkyl, [4-(5-[C_1-C_4 alkyl]-1,3-dioxolen-2-onyl)]methyl, acetonyl, indanyl and pyridyl.

Preferred biolabile ester-forming groups are methyl, ethyl, (3-cyclohexyl)propyl, (3-phenyl)propyl, pivaloyloxymethyl, 1-(cyclohexylacetoxy)ethyl, 1-(cyclohexylcarboxy)ethyl, 1-(2-indanylcarboxy)ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)-ethyl and [4-(5-methyl-1,3-dioxolen-2-onyl)]methyl.

The invention also includes amide derivatives (wherein either

or both of OR^1 and OR^2 are replaced by NH_2). Such compounds are also bioprecursors to the dicarboxylic acids and their suitability too may be assessed as indicated above.

The compounds of the formula (I) contain three or more asymmetric centres and thus they can exist as enantiomers or diastereoisomers. The invention includes both the separated individual isomers as well as mixtures of isomers. The preferred stereoisomers are those derived from either (S)-proline or 4(R)-hydroxy-(S)-proline, in which each of the terminal carboxylic acid/ester/amide groups is attached to an asymmetric carbon atom of (S)-configuration.

Also included in the invention are radiolabelled derivatives of compounds of the formula (I) which are suitable for biological studies.

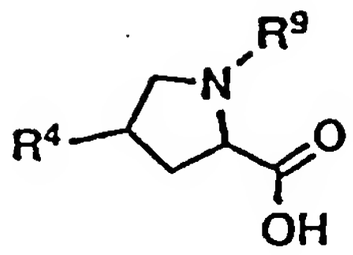
The pharmaceutically acceptable salts of the compounds of formula (I) containing an acidic centre are those formed with bases which form non-toxic salts. Examples include the alkali or alkaline earth metal salts such as the sodium, potassium or calcium salts, or salts with amines such as diethylamine. Compounds having a basic centre can also form acid addition salts with pharmaceutically acceptable acids. Examples include the hydrochloride, hydrobromide, sulphate or bisulphate, phosphate or hydrogen phosphate, acetate, benzoate, citrate, tartrate, lactate, fumarate, maleate, succinate, gluconate, methanesulphonate, benzenesulphonate and p-toluenesulphonate salts.

Preferred compounds are prodrug mono or diesters of compounds of the formula (I) wherein R^3 is benzyloxymethyl (O-benzyl serine

derivates), 1-(2-butenyl)oxymethyl, 1-(4-methoxy-2-butenyl)oxymethyl or 2-chloro-2-propenyloxymethyl. R^4 is preferably H. Preferred mono-esters are those wherein R^1 is H and R^2 is methyl, (3-phenyl)propyl or (3-cyclohexyl)propyl, and preferred diesters are those wherein R^1 is pivaloyloxymethyl, 1-(cyclohexylacetoxy)-ethyl, 1-(cyclohexylcarboxy)ethyl, 1-(2-indanylcarboxy)ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl or [4-(5-methyl-1,3-dioxolen-2-onyl]methyl, and R^2 is ethyl.

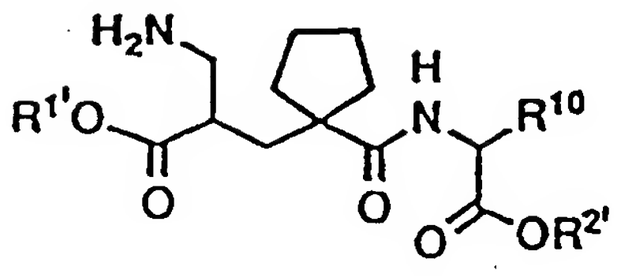
It will be appreciated from the foregoing discussion that the biologically active species <u>in vivo</u> are the diacids, that is compounds of the formula (I) wherein both R^1 and R^2 are H, and R^3 and R^4 are as previously defined for the formula (I). Thus these diacids form a further preferred aspect of the invention.

The compounds of the formula (I) can be prepared by a number of methods using the coupling and protective procedures of amino-acid chemistry. One procedure involves coupling of a suitably N-protected proline or 4-hydroxyproline derivative of the formula (II), wherein R^4 is as previously defined and R^9 is a conventional amino acid N-protecting group such as t-butoxycarbonyl, 2,2,2-trichloroethoxycarbonyl or benzyloxycarbonyl, with an amine of the formula (III), wherein $R^{1'}$ and $R^{2'}$ are as previously defined for R^1 and R^2 respectively but are not H, and R^{10} is as defined for R^3 with any reactive groups therein optionally protected, to provide a compound of the formula (IV) as shown in the following reaction scheme:

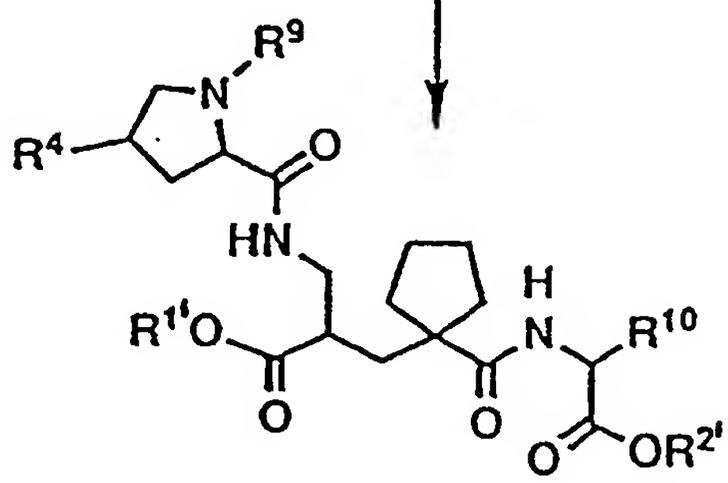


(II)

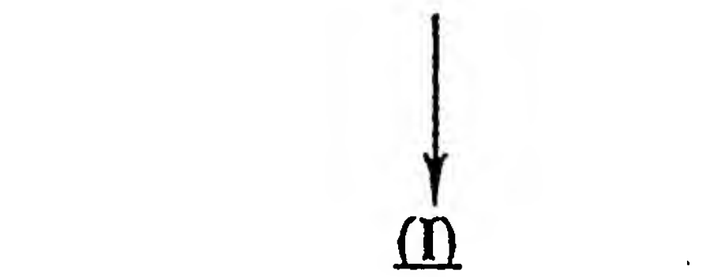
+



(III)



(IV)

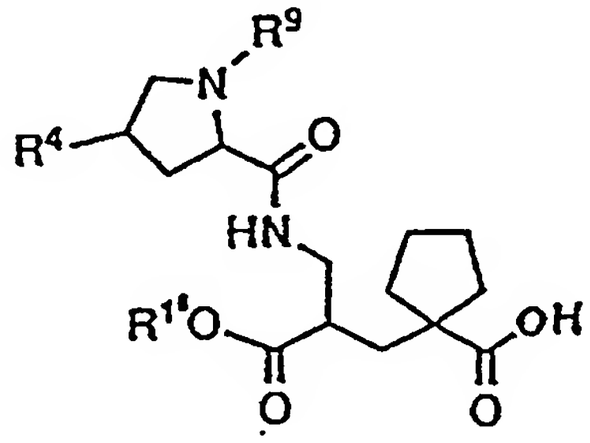


(I)

The reaction of the compounds of formula (II) and (III) is achieved using conventional amide coupling techniques. Thus in one process the reaction is achieved with the reactants dissolved in an organic solvent, e.g. dichloromethane, using a diimide condensing agent, for example 1-ethyl-3-(dimethylaminopropyl)-carbodiimide, or N,N'-dicyclohexylcarbodiimide, advantageously in the presence of 1-hydroxybenzotriazole and an organic base such as N-methylmorpholine. The reaction is generally complete after a period of from 12 to 24 hours at room temperature and the product is then isolated by conventional procedures, i.e. by washing with water, or filtration, to remove the urea by-product and

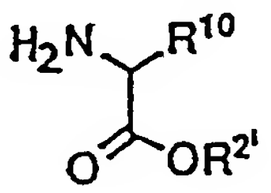
evaporation of the solvent. The product may be further purified by crystallisation or chromatography if necessary.

In an alternative analogous procedure, the diester products of formula (IV) may be obtained by coupling compounds of formulae:



(V)

+



(VI)

and the reaction is achieved following similar procedures to those described above.

The diesters of formula (IV) are subsequently deprotected to give the diester, monoester or diacid derivatives of formula (I). The conditions used will depend on the precise nature of the groups $R^{1'}$ and $R^{2'}$ in the compound of formula (IV) and a number of variations is possible. Thus, for example, when one of $R^{1'}$ and $R^{2'}$ is t-butyl and the other is alkyl, treatment of the compound of formula (IV) with hydrogen chloride or trifluoroacetic acid yields a monoalkyl ester of formula (I) wherein one of $R^{1'}$ and $R^{2'}$ is H and the other is alkyl. Alternatively, when both of $R^{1'}$ and $R^{2'}$ are t-butyl, said acid deprotection affords a diacid of formula (I) wherein R^1 and R^2 are both H. As an alternative carboxylic acid protecting group, benzyl may be employed instead of t-butyl. In such cases, catalytic hydrogenation removes the benzyl groups(s) to furnish either the monoester or diacid as

required.

A further variation is that in which a monoester of formula (I), wherein R^1 is H and R^2 is a biolabile ester-forming group, is converted to a diacid of formula (I), wherein both R^1 and R^2 are H, by base hydrolysis, e.g. using an aqueous sodium or potassium hydroxide medium.

Amides wherein either or both of OR^1 and OR^2 are replaced by NH_2 are obtained by starting with the appropriate amide derivative corresponding with formula (III), (V) or (VI) in the coupling step, that is wherein either of $OR^{1'}$ or $OR^{2'}$ is NH_2 or both of $OR^{1'}$ and $OR^{2'}$ are NH_2.

Depending on the protection/deprotection strategy employed, further conventional deprotection steps may be required to remove R^9 and/or any protecting groups present in R^{10}.

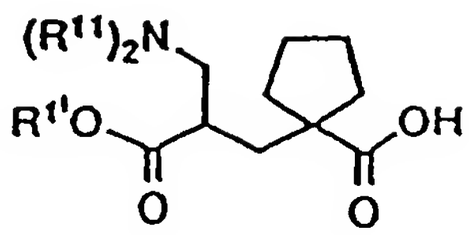
Required diester prodrugs which are not directly accessible from compounds of formula (IV) may be obtained from monoesters of formula (IV) wherein either $R^{1'}$ or $R^{2'}$ is H. This may be achieved for example by alkylation of an alkali metal, preferably caesium, salt of the monoacid with the required alkyl halide, preferably bromide or iodide, or by coupling of the monoacid with an alcohol or phenol by conventional techniques as described above. Further deprotection steps, e.g. to remove R^9 and/or any protecting group contained in R^{10}, are carried out as appropriate to afford compounds of formula (I) wherein neither R^1 nor R^2 is H.

Thus certain novel compounds of the formula (IV), wherein $R^{1'}$ and $R^{2'}$ are each independently selected from t-butyl or benzyl, are useful intermediates for the preparation of compounds of the formula (I) and also form part of the invention.

The compounds of formula (I) may be isolated as, for example, hydrochloride or sodium salts directly from the previous deprotection step. Alternatively, they may be converted to other pharmaceutically acceptable acid addition, alkali metal or alkaline earth metal salts by routine procedures.

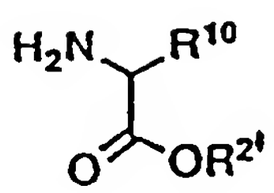
Appropriate coupling, protection and deprotection methods for all of the above steps and alternative variations and procedures will be well known to those skilled in the art by reference to relevant text-books and to the examples provided hereafter.

The proline derivatives of formula (II) are either commercially available or preparable by standard methods in accordance with literature precedent. The amines of formula (III) may be prepared by analogy with processes described in EP-A-0358398 using the aminomethylglutaric acid derivative of formula (VII), wherein R^{11} is benzyl or 1(S)-phenylethyl and $R^{1'}$ is as previously defined, and the appropriate α-amino ester of formula (VI) , wherein R^{10} and $R^{2'}$ are as previously defined, to afford the coupled product of the formula (VIII), followed by catalytic hydrogenolytic removal of the R^{11} groups as shown in the following reaction scheme:



(VII)

+



(VI)

(VIII)

(III)

The compounds of formula (V) are preparable according to processes described in EP-A-0358398, by coupling the sodium salt of 1-(2-t-butoxycarbonyl-3-aminopropyl)cyclopentane carboxylic acid with the proline fragment (II).

The novel α-amino esters of formula (VI) may be prepared from commercially available N-protected α-amino acid derivatives such as those of glycine, serine or tyrosine by established methods in accordance with literature precedent. For example, standard alkylation of the serine alcoholic hydroxyl group provides ether derivatives, whilst Mitsunobu or Heck modification of the tyrosine phenolic hydroxyl group or its trifluoromethanesulphonyl

derivative respectively leads to a variety of 4-alkoxy or 4-alkyl phenylalanine derivatives, which are also obtainable by C-benzylation of glycine derivatives using the asymmetric alkylation procedure developed by O'Donnell et al. Subsequent N-deprotection then affords the compounds of formula (VI).

As previously mentioned, the prodrugs of the invention offer an oral bioavailability advantage in the systemic delivery of the potent, dual inhibitor diacids of the formula (I) derived therefrom wherein R^1 and R^2 are H. These diacids are potent inhibitors of the neutral endopeptidase (E.C.3.4.24.11). This enzyme is involved in the breakdown of a number of peptide hormones including, in particular, the breakdown of atrial natriuretic factor (ANF). Thus the diacids of the invention, by preventing the degradation of ANF by endopeptidase E.C.3.4.24.11, can potentiate its biological effects and the compounds are thus diuretic, natriuretic and antihypertensive agents of utility in a number of disorders including hypertension, heart failure, angina, renal insufficiency, premenstrual syndrome, cyclical oedema, Menieres disease, hyperaldosteronism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF, the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of asthma, inflammation, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity, gastrointestinal disorders (especially diarrhoea and

irritable bowel syndrome), the modulation of gastric acid secretion and the treatment of hyperreninaemia.

Activity against neutral endopeptidase E.C.3.4.24.11 is assessed using a procedure based on the assay described by J. T. Gafford, R. A. Skidgel, E. G. Erdos and L. B. Hersh, Biochemistry, 1983, 32, 3265-3271. The method involves determining the concentration of compound required to reduce by 50% the extent of release of radiolabelled hippuric acid from hippuryl-L-phenyl-alanyl-L-arginine by a neutral endopeptidase preparation from rat kidney.

The diacids of the invention are also inhibitors of angiotensin converting enzyme. As such they are useful in treating a further variety of conditions for which ACE inhibitors are known to be useful including limitation of ischaemic damage to the myocardium, protection of the kidney against hyperfiltration damage, prevention or reversal of left ventricular hypertrophy, memory enhancement, control of cognitive function, dementia, and preventing reocclusion following coronary angioplasty or coronary artery bypass surgery. Their activity against this enzyme is assessed using a modified procedure based on the assay described by M.S. Rohrbach, Anal. Biochem., 1978, 84, 272. The method involves determining the concentration of compound required to reduce by 50% the extent of release of radiolabelled hippuric acid from hippuryl-L-histidyl-L-leucine by angiotensin converting enzyme isolated from rat kidney.

Inhibitory activity is also measured in vivo following intravenous injection to anaesthetised rats using the methods described by I. L. Natoff et al., Journal of Pharmacological

Methods, 1981, 5, 305 and by D. M. Gross et al., J. Pharmacol. Exp. Ther., 1981, 216, 552. The dose of inhibitor required to reduce the pressor response produced by intravenous injection of angiotensin I (50 ng bolus) by 50% is determined.

The activity of the diacids as diuretic agents is determined by measuring their ability to increase urine output and sodium ion excretion in saline loaded conscious mice. In this test, male mice (Charles River CD1, 22-28 g) are acclimatised and starved overnight in bowls. The mice are dosed intravenously via the tail vein, with the test compound dissolved in a volume of saline solution equivalent to 2.5% of body weight. Urine samples are collected each hour for two hours in pre-weighed tubes and analysed for electrolyte concentration. Urine volumes and sodium ion concentrations from the test animals are compared with those of a control group which received only saline.

The antihypertensive activity of the prodrugs of the invention and the diacids derived therefrom is evaluated by measuring the fall in blood pressure, following oral or intravenous administration respectively, to salt-depleted, diuretic primed, spontaneously hypertensive rats, salt-depleted renally hypertensive dogs, or DOCA/salt hypertensive rats.

The systemic bioavailability of diacid obtained after oral administration of a prodrug of the invention is determined, e.g. in rat, by measuring the fraction of the biologically active dose recovered via the urine (measured by in vitro assay of neutral endopeptidase activity as previously described) and comparing it with the corresponding fraction after an equivalent dose of the corresponding diacid, when administered by the intravenous route.

Alternatively plasma concentrations of diacid are measured in dog following oral administration of the prodrug and again compared with the corresponding values obtained after the intravenous administration of an equivalent dose of the corresponding diacid.

For administration to man in the curative or propylactic treatment of hypertension, congestive heart failure or renal insufficiency, oral dosages of the compounds of the invention will generally be in the range of from 3-1500 mg daily for an average adult patient (70 kg). Thus for a typical adult patient, individual tablets or capsules contain from 1 to 500 mg of active compound, in a suitable pharmaceutically acceptable vehicle or carrier for administration singly, or in multiple doses, once or several times a day. Dosages for intravenous administration would typically be within the range 1 to 500 mg per single dose as required. In practice the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

For human use, the compounds of the formula (I) can be administered alone, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose,

or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or glucose to make the solution isotonic with blood.

The compounds may be co-administered with other agents as may be beneficial for the control of blood pressure or the treatment of cardiac conditions or renal insufficiency. Thus, for example, they may be co-administered with digitalis or another cardiac stimulant drug, an alpha-blocker, a beta-blocker, exogenous ANF, a potassium channel activator or another diuretic agent, as shall be determined by the physician as appropriate to the particular patient or disease state.

Thus, in a further aspect, the invention provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

The invention also includes a compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either, for use in medicine.

The invention further includes the use of a compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either, for the manufacture of a medicament for the treatment of hypertension, heart failure or renal insufficiency.

The invention yet further includes a method for the propylactic or curative treatment of hypertension, heart failure or renal insufficiency in a human being, which comprises administering to said human being an effective amount of a compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either.

The preparation of the compounds of the invention and of the intermediates for use in their preparation are illustrated by the following Examples and Preparations. The purity of the compounds was routinely monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} plates and the following solvent systems (SS):

1 hexane/ethyl acetate, 4:1

2 hexane/diethyl ether, 1:1

3 hexane/ethyl acetate, 1:1

4 hexane/diethyl ether, 6:4

5 hexane/diethyl ether, 1:4

6 hexane/diethyl ether, 3:7

7 ethyl acetate/ethanol, 9:1

8 ethyl acetate

9 dichloromethane/methanol/acetic acid, 80:20:1

10 isobutyl methyl ketone/acetic acid/water, 2:1:1 (upper phase)

11 hexane/ethyl acetate, 1:4

12 ethyl acetate/ethanol, 19:1

13 hexane/diethyl ether, 4:1

14 dichloromethane/methanol, 9:1

15 diethyl ether/dichloromethane, 1:1

16 diethyl ether

17 hexane/ethyl acetate, 6:4

18 hexane/diethyl ether, 4:6

19 dichloromethane/methanol/ammonia, 90:10:1

20 hexane/2-propanol/ammonia, 90:10:0.5

21 dichloromethane/ethanol/acetic acid, 90:10:1

22 dichloromethane/methanol/acetic acid, 90:10:1

23 n-butanol/water/acetic acid, 12/5/3

24 dichloromethane/methanol/acetic acid, 40:10:1

25 ethyl acetate/toluene, 1:1

26 dichloromethane/methanol/acetic acid/hexane, 90:10:1:150

^{1}H-Nuclear magnetic resonance (nmr) spectra were recorded using a Nicolet QE-300 or Brucker AC-300 spectrometer and were in all cases consistent with the structures of the compounds described hereinafter.

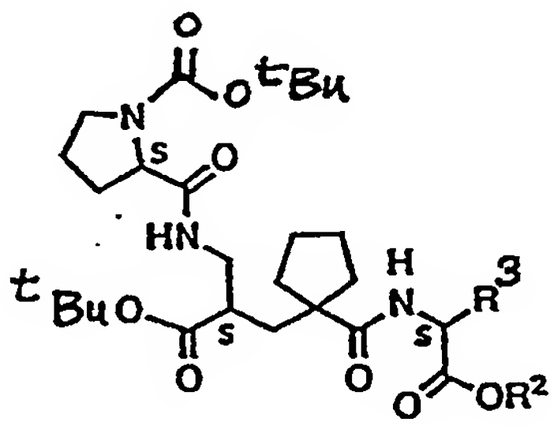
EXAMPLE 1

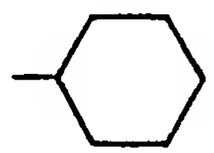
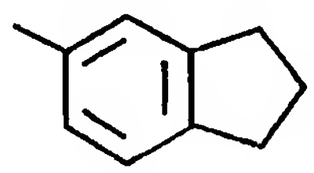
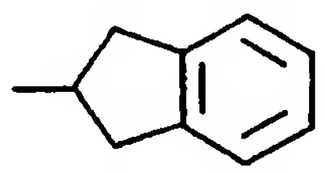
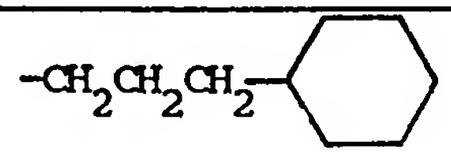
N-[1-{3-[N-t-Butoxycarbonyl-(S)-prolylamino]-2(S)-t-butoxy-carbonylpropyl}cyclopentanecarbonyl]-O-benzyl-(S)-serine methyl ester

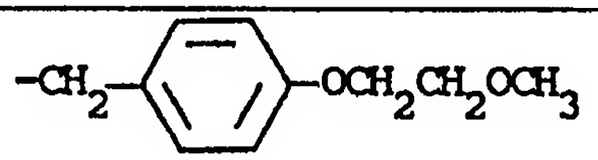
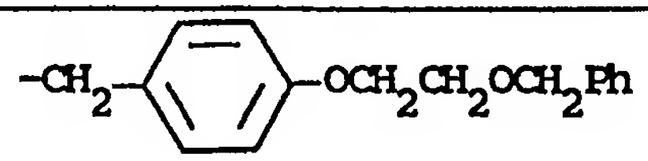
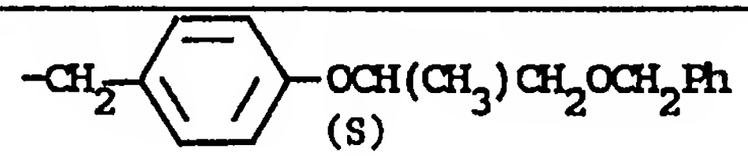
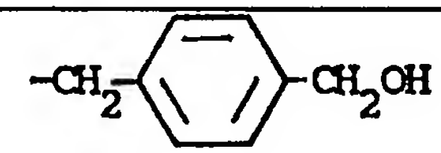
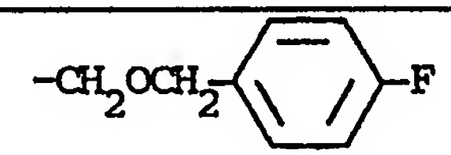
To a stirred, ice-cold solution of 1-{3-[N-t-butoxy-carbonyl-(S)-prolylamino]-2(S)-t-butoxycarbonylpropyl}cyclopentane carboxylic acid (Preparation 80, 351 mg, 0.75 mmol) in dichloromethane (15 ml) were added, sequentially, 1-hydroxybenzotriazole (122 mg, 0.90 mmol), N-methylmorpholine (265 mg, 2.62 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (287 mg, 1.50 mmol) and, 0.25 hour later, O-benzyl-(S)-serine methyl ester hydrochloride (202 mg, 0.82 mmol). The ice bath was removed, stirring continued for 24 hours, then the reaction mixture was evaporated under vacuum. The residue was partitioned between ethyl acetate (100 ml) and 2M hydrochloric acid (50 ml), then the organic phase separated, washed successively with 2M hydrochloric acid (2 x 30 ml), saturated aqueous sodium bicarbonate solution (30 ml) and saturated brine (30 ml), dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate provided a foam (546 mg) which was purified by chromatography on silica gel, using 30% ethyl acetate in hexane as eluent, to afford the title compound as a white foam (409 mg, 83%). Rf 0.54 (SS 15) and 0.24 (SS 3), $[\alpha]_D^{25}$ -18° (c = 0.1, MeOH). Found: C,63.35; H,8.12; N,6.23. $C_{35}H_{53}N_3O_9$ requires C,63.71; H,8.10; N,6.37%.

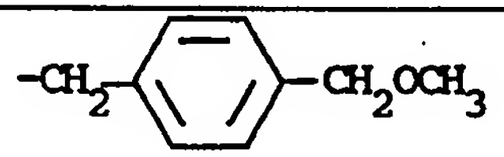
EXAMPLES 2-40

The following Examples were obtained according to the method of Example 1 using 1-(3-[N-t-butoxycarbonyl-(S)-prolylamino-2(S)-t-butoxycarbonylpropyl)cyclopentane carboxylic acid and the appropriate α-amino ester of formula (VI) from the Preparations section.

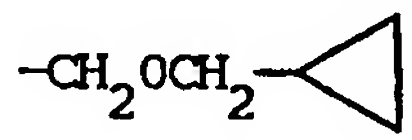
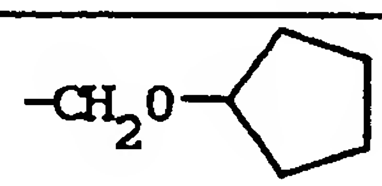
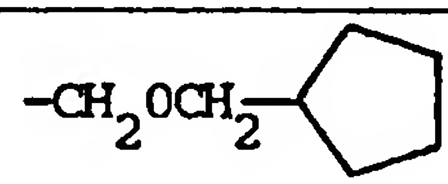


Example No.	R^3	R^2	Rf	Analysis % C (theoretical in brackets)	H	N
2	$-CH_2OCH_2Ph$	$-CH(CH_2CH_3)_2$	0.45 (SS 3)	65.28 (65.43	8.59 8.59	5.86 5.87)
3	$-CH(CH_3)OCH_2Ph$ (R)	$-CH_3$	0.57 (SS 16)			
4	$-CH_2OCH_2Ph$		0.38 (SS 16)	65.78 (65.99	8.30 8.45	5.59 5.77)
5	$-CH_2OCH_2Ph$		0.66 (SS 8)	67.26 (67.77	7.96 7.80	5.47 5.51)
6	$-CH_2OCH_2Ph$		0.44 (SS 3)	67.58 (67.77	7.83 7.80	5.47 5.51)
7	$-CH_2OCH_2Ph$		0.67 (SS 16)	67.37 (67.07	8.62 8.77	5.26 5.46)

8	$-CH_2OCH_2Ph$	$-(CH_2)_3Ph$	0.64 (SS 16)	67.64 (67.60	8.06 8.05	5.50 5.50)
9		^tBu	0.40 (SS 17)	62.43 (62.80	8.39 8.69	5.72 5.49)a
10		^tBu	0.56 (SS 3)	67.51 (67.21	7.89 8.21	4.84 5.11)
11	 (S)	^tBu	0.22 (SS 18)	67.53 (67.52	8.59 8.32	4.87 5.03)
12		^tBu	0.22 (SS 6)	64.86 (65.03	8.41 8.47	5.92 5.99)
13		$-(CH_2)_3Ph$		66.92 (66.82	7.90 8.08	5.45 5.44)b
14		$-(CH_2)_3Ph$	0.54 (SS 3)	65.84 (66.05	7.80 7.73	5.31 5.37)

15	$-CH_2OCH_2C(Cl)=CH_2$	$-(CH_2)_3Ph$	0.65 (SS 3)			
16	$-CH_2CH_2OCH_2Ph$	$-(CH_2)_3Ph$	0.65 (SS 3)			
17		^tBu	0.65 (SS 5)	65.24 (65.43	8.59 8.59	5.72 5.87)
18	$-CH_2OCH_2CH=CHCH_2OCH_3$ (E)	$-(CH_2)_3Ph$	0.79 (SS 16)	64.94 (64.97	8.25 8.38	5.41 5.54)
19	$-CH_2OCH_2CH=CHCH_3$ (E)	$-(CH_2)_3Ph$	0.45 (SS 3)	66.22 (66.00	8.34 8.45	5.58 5.77)
20	$-CH_2OCH_2CH=CH_2$	$-(CH_2)_3Ph$	0.39 (SS 3)	61.59 (61.61	8.69 8.56	6.86 6.74)
21	$-CH_2O(CH_2)_3CH_3$	$-CH_2Ph$	-		-	

22	$-CH_2CH_2O(CH_2)_2CH_3$	$-C_2H_5$	0.6 (SS 5)	62.19 (61.95	8.89 8.98	6.46 6.57)
23	$-CH_2O(CH_2)_2CH_3$	$-C_2H_5$	0.38 (SS 3)	61.97 (61.41	8.62 8.86	6.67 6.71)
24	$-CH_2\underset{E}{CH=CH}CH_2OCH_2CH_3$	$-CH_3$	0.26 (SS 3)	61.99 (62.14	8.48 8.69	6.45 6.59)
25	$-CH_2OCH_2C\equiv CCH_3$	$-CH_2Ph$	0.79 (SS 16)	-		
26	$-CH_2O(CH_2)_3OCH_3$	$-(CH_2)_3Ph$	0.34 (SS 3)	64.37 (64.40	8.27 8.51	5.58 5.63)
27	$-CH_2OCH_2\underset{\|}{\overset{CH_2}{C}}CH_2OCH_3$	$-(CH_2)_3Ph$	0.65 (SS 3)	64.36 (64.41	7.97 8.31	5.42 5.48)c
28	$-CH_2OCH_2C\equiv CH$	$-(CH_2)_3Ph$	0.46 (SS 3)	66.04 (65.80	8.14 8.07	5.96 5.90)

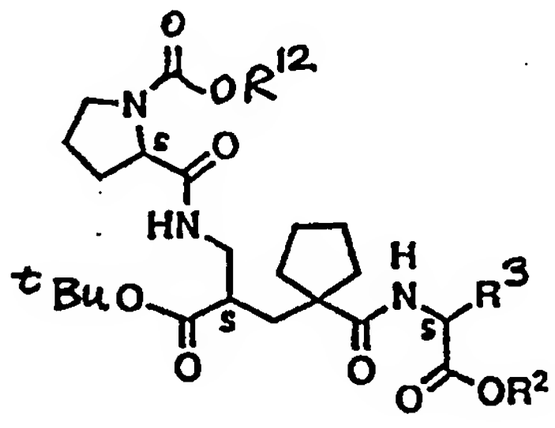
29		$-CH_2Ph$	0.09 (SS 26)		-	
30	$-CH_2OCH_2CF_3$	$-CH_2Ph$	0.09 (SS 26)		-	
31	$-CH_2OCH(CH_3)_2$	$-CH_2Ph$	0.09 (SS 26)		-	
32	$-CH_2OCH(CH_3)CF_3$	$-CH_2Ph$	0.09 (SS 26)	55.44 (55.29	7.03 7.42	6.31 6.45)
33		$-CH_2Ph$	0.10 (SS 26)		-	
34	$-CH_2OCH_2C(=CH_2)CH_3$	$-(CH_2)_3Ph$	0.74 (16)	65.32 (65.40	8.42 8.38	5.63 5.71)c
35		$-C_2H_5$	0.41 (SS 3)	62.91 (63.13	8.95 8.93	6.28 6.31)

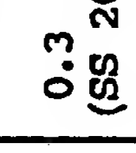
36	$-CH_2OCH_2CH_2CH(CH_3)_2$	$-C_2H_5$	0.46 (SS 3)	62.31 (62.46	8.89 9.09	6.35 6.43)
37	$-CH_2OCH_2CH=CHCH_2OCH_3$ E	$-C_2H_5$	0.50 (SS 16)	61.04 (61.15	8.47 8.60	6.25 6.29)
38	$-CH_2OCH_2CH=CHCH_3$ E	$-C_2H_5$	0.80 (SS 16)	62.11 (62.14	8.42 8.69	6.60 6.59)
39	$-CH_2OCH_2Ph$	$-C_2H_5$	0.36 (SS 3)	64.16 (64.02	8.23 8.00	6.24 6.15)
40	$-(CH_2)_4OCH_3$	$-CH_3$	0.35 (SS 3)	61.19 (61.42	8.78 8.86	6.67 6.71)

a. H_2O
b. $0.50\ H_2O$
c. $0.1\ CH_2Cl_2$

EXAMPLES 41-45

The following compounds were prepared following the coupling procedure of Example 1 but using the appropriate N-protected proline derivative of formula (V) (see Preparations 81 and 82) and coupling to the appropriate α-amino ester or amide derivative of formula (VI).



Example No.	R^3	OR^2	R^{12}	Rf	Analysis % (theoretical in brackets) C	H	N
41	$-CH_2OCH_3$	$-O(CH_2)_3Ph$	$PhCH_2-$	0.28 (SS 20)	66.83 (66.55	7.74 7.68	5.76 5.82)
42		$-O(CH_2)_3Ph$	$PhCH_2-$	0.3 (SS 20)	67.62 (67.78	7.78 7.80	5.39 5.31)
43	$-CH_2OCH_2Ph$	$-OC_2H_5$	$PhCH_2-$	0.51 (SS 16)	65.56 (65.50	7.34 7.58	5.86 5.87)d
44	$-CH_2OCH_2Ph$	$-OC_2H_5$	Cl_3CCH_2-	0.28 (SS 3)	54.13 (54.51	6.48 6.46	5.73 5.61)
45	$-CH_2OCH_2Ph$	$-NH_2$	Cl_3CCH_2-	0.58 (SS 8)	52.03 (52.18	6.21 6.43	7.57 7.77)e

d. 0.40 H_2O
e. 0.25 CH_2Cl_2

EXAMPLE 46

N-{1-[3-Benzyloxycarbonylamino-2(S)-t-butoxycarbonylpropyl]-cyclopentanecarbonyl}-O-benzyl-(S)-serine (3-phenyl)propyl ester

This was obtained by the procedure of Example 1 using 1-[3-benzyloxycarbonylamino-2(S)-t-butoxycarbonylpropyl]cyclopentane carboxylic acid (Preparation 83) and the product of Preparation 38 to provide the title compound, Rf 0.52 (SS 3). Found: C,67.25; H,7.44; N,3.89. $C_{41}H_{52}N_2O_8$; 1.50 H_2O requires C,67.65; H,7.61; N,3.85%.

EXAMPLE 47

N-{1-[3-Amino-2(S)-t-butoxycarbonylpropyl]cyclopentanecarbonyl}-O-benzyl-(S)-serine (3-phenyl)propyl ester

This was obtained from Example 46 by Method C (catalytic hydrogenation - see Preparation 37) to provide the title compound, Rf 0.67 (SS 10). Found: C,69.54; H,8.45; N,4.66. $C_{33}H_{46}N_2O_6$ requires C,69.93; H,8.18; N,4.94%.

EXAMPLE 48

N-[1-{2(S)-t-Butoxycarbonyl-3-[N-t-butoxycarbonyl-4(R)-hydroxy-(S)-prolylamino]propyl}cyclopentanecarbonyl]-O-benzyl-(S)-serine (3-phenyl)propyl ester

This was obtained from N-t-butoxycarbonyl-4(R)-hydroxy-(S)-proline and Example 47 using the coupling methodology of Example 1, Rf 0.61 (SS 8), Found: C,65.91; H,7.73; N,5.31. $C_{43}H_{61}N_3O_{10}$ requires C,66.22; H,7.88; N,5.39%.

EXAMPLE 49

N-[1-{3-(N-Benzyloxycarbonyl-(S)-prolylamino)-2(S)-carboxy-propyl}-1-cyclopentanecarbonyl]-O-benzyl-(S)-serine ethyl ester

The title compound was prepared from Example 43, by trifluoroacetic acid deprotection (see deprotection Method A, Example 76) and was obtained as a white foam, Rf 0.68 (SS 10). Found: C,64.92; H,6.90; N,6.13. $C_{35}H_{45}N_3O_9$ requires C,64.49; H,6.96; N,6.45%.

EXAMPLE 50

N-[1-{3-(N-(2,2,2-Trichloroethoxycarbonyl-(S)-prolylamino)-2-(S)-carboxypropyl}-1-cyclopentanecarbonyl]-O-benzyl-(S)-serine ethyl ester

Prepared from Example 44 as described above to give the title product as a white foam, Rf 0.39 (SS 14). Found C,51.31; H,5.61; N,5.86. $C_{30}H_{40}N_3Cl_3O_9$; 0.5 H_2O requires C,51.33; H,5.89; N,5.98%.

EXAMPLE 51

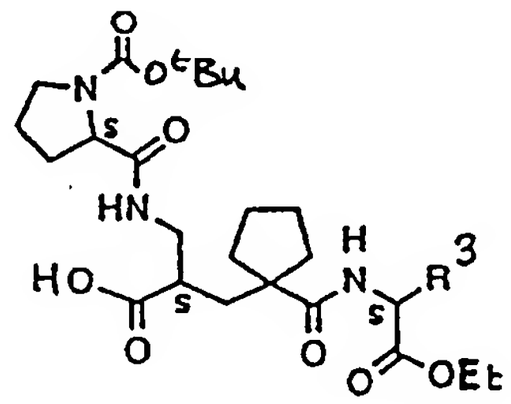
N-[1-{3-(N-t-Butoxycarbonyl-(S)-prolylamino)-2(S)-carboxy-propyl}-1-cyclopentanecarbonyl]-O-[trans-4-methoxybut-2-enyl]-(S)-serine ethyl ester

A stirred solution of N-[1-{3-(N-t-butoxycarbonyl-(S)-prolylamino)-2(S)-t-butoxycarbonylpropyl}-1-cyclopentane-carbonyl}-O-[trans-4-methoxybut-2-enyl]-(S)-serine ethyl ester (1.13 g, 1.69 mmol) in dry dichloromethane (20 ml) at -5°C was saturated with anhydrous hydrogen chloride. After five hours the reaction was degassed with nitrogen, the solvent evaporated under

vacuum and the residual foam dried azeotropically with dichloromethane. This crude product was dissolved in a solution of sodium bicarbonate (0.427 g, 5.076 mmole) in water (20 ml) and the resulting solution cooled to 15°C. A solution of di-t-butyldicarbonate (0.739 g, 3.384 mmol) in dioxan (20 ml) was added dropwise with stirring and the resulting mixture allowed to warm to room temperature. After eighteen hours the reaction was evaporated under vacuum to low volume, diethyl ether (20 ml) and water (20 ml) were added and the aqueous layer separated, washed with diethyl ether and then acidified to pH 2 with 2N hydrochloric acid. The crude product was extracted with ethyl acetate (3 x 30 ml) and the combined extracts were washed with brine, dried ($MgSO_4$) and evaporated under vacuum. Azeotropic treatment of the residue with dichloromethane gave the required product as a colourless foam (960 mg, 92%), Rf 0.55 (SS 22). Found: C,58.62; H,7.97; N,7.04. $C_{30}H_{49}N_3O_{10}$ requires C,58.90; H,8.07; N,6.87%.

EXAMPLES 52-53

The following compounds were prepared from Examples 38 and 39 using the procedure described above for Example 51.



Example No.	R^3	Rf	Analysis % (theoretical in brackets) C	H	N
52	$-CH_2OCH_2CH=CHCH_3$ E	0.56 (SS 22)	59.24 (59.41	8.16 8.17	7.35 7.17)a
53	$-CH_2OCH_2Ph$	0.53 (SS 22)	51.31 (51.33	5.61 5.89	5.86 5.98)b

a. 0.25 H_2O b. 0.50 H_2O

EXAMPLE 54

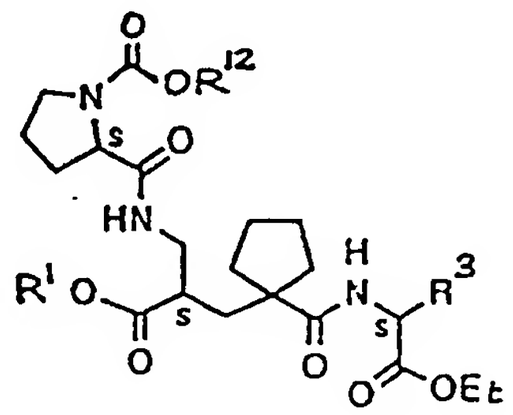
N-[1-{3-(N-Benzyloxycarbonyl-(S)-prolylamino)-2(S)-pivaloyloxy-methoxycarbonylpropyl}-1-cyclopentanecarbonyl]-O-benzyl-(S)-serine ethyl ester

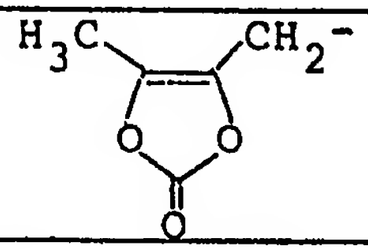
N-[1-{3-(N-Benzyloxycarbonyl-(S)-prolylamino)-2(S)-carboxy-propyl}-1-cyclopentanecarbonyl]-O-benzyl-(S)-serine ethyl ester (Example 49, 0.648 g, 0.99 mmol) was dissolved in acetonitrile (10 ml) and water (5 ml) added. Aqueous caesium carbonate (12%) was added until the pH was 8, the resulting solution evaporated under vacuum and then the residue azeotroped with toluene (4 x 10 ml). The resulting foam was dissolved in N,N-dimethylacetamide (5 ml)

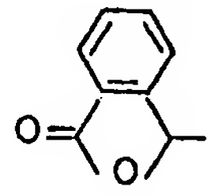
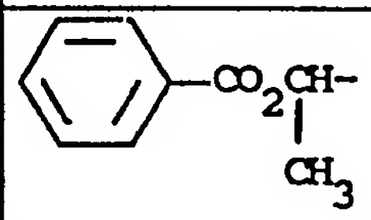
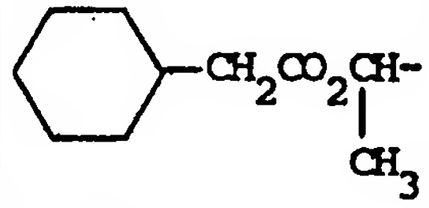
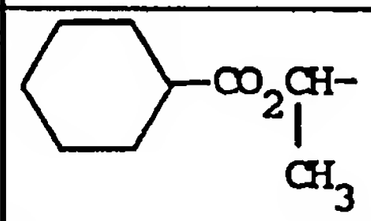
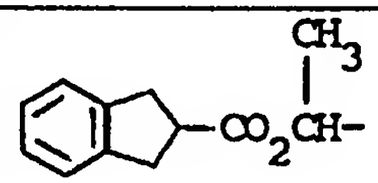
and pivaloyloxymethylchloride (0.298 g, 1.98 mmol) added to the stirred solution. After 16 hours at room temperature the solvent was removed under vacuum and the residue partitioned between diethyl ether (100 ml) and 2M hydrochloric acid (50 ml). The ether layer was separated, washed successively with 2M hydrochloric acid (2 x 25 ml) and saturated brine (25 ml), dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate afforded the crude product which was purified by column chromatography (40 g silica, eluent 30% ethyl acetate in hexane) to give the title compound as a white foam, Rf 0.61 (SS 15). Found C,64.50; H,7.33; N,5.43. $C_{41}H_{55}N_3O_{11}$ requires C,64.29; H,7.24; N,5.49%.

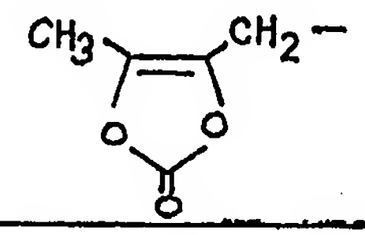
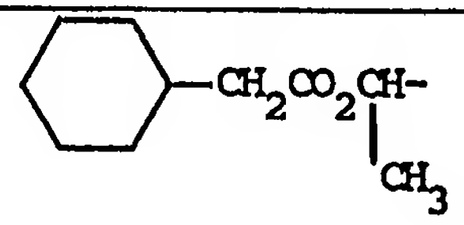
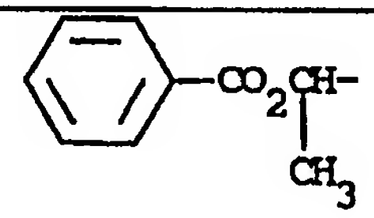
EXAMPLES 55-71

The following Examples were prepared using the procedure described above by reaction of the caesium salt of the appropriate monoester with the appropriate chloride of formula R^1-Cl, except that for Example 67 the corresponding alkyl iodide was used and for Examples 59, 61 and 68 the corresponding alkyl bromides were used.



Example No.	R^3	R^1	R^{12}	Rf	Analysis % C H N (theoretical in brackets)
55	$-CH_2OCH_2Ph$	$(CH_3)_2CHCO_2CH(CH_3)-$	Cl_3CCH_2-	0.68 (SS 8)	53.77 6.23 5.22 (53.57 6.24 5.20)
56	$-CH_2OCH_2Ph$	$CH_3(CH_2)_2CO_2CH(CH_3)-$	Cl_3CCH_2-	0.66 (SS 8)	53.75 6.35 5.18 (53.57 6.24 5.20)
57	$-CH_2OCH_2Ph$	$CH_3CH_2OCO_2CH(CH_3)-$	Cl_3CCH_2-	0.19 (SS 3)	52.50 6.04 5.13 (51.95 5.97 5.19)
58	$-CH_2OCH_2Ph$	CH_3COCH_2	Cl_3CCH_2-	0.51 (SS 8)	-
59	$-CH_2OCH_2Ph$		t_{Bu}	0.48 (SS 8)	60.39 7.06 5.65 (60.55 7.01 5.71)a
60	$-CH_2OCH_2Ph$	$CH_3CH_2CO_2C(CH_3)_2-$	Cl_3CCH_2-	0.36 (SS 25)	-

61	$-CH_2OCH_2Ph$		Cl_3CCH_2-	0.22 (SS 16)	54.72 (54.54	5.13 5.29	4.45 4.96)b
62	$-CH_2OCH_2CH=CHCH_2OCH_3$ (E)	$(CH_3)_3CCO_2CH_2-$	t_{Bu}	0.55 (SS 16)	60.09 (59.57	8.28 8.19	5.71 5.79)
63	$-CH_2OCH_2CH=CHCH_2OCH_3$ (E)		t_{Bu}	0.35, 0.40 (SS 16)	61.33 (61.64	7.23 7.56	5.19 5.53)
64	$-CH_2OCH_2CH=CHCH_2OCH_3$ (E)		t_{Bu}	0.60, 0.65 (SS 16)	61.50 (61.60	8.53 8.40	5.03 5.39)
65	$-CH_2OCH_2CH=CHCH_3$ (E)		t_{Bu}	0.57, 0.51 (SS 16)	61.87 (62.02	8.15 8.35	5.70 5.71)
66	$-CH_2OCH_2CH=CHCH_3$ (E)		t_{Bu}	0.55, 0.65 (SS 16)	62.37 (62.36	7.33 7.55	5.09 5.28)c
67	$-CH_2OCH_2CH=CHCH_3$ (E)	n_{Bu}	t_{Bu}	0.40 (SS 3)	62.66 (62.14	8.33 8.69	6.78 6.59)

68	$-CH_2OCH_2CH=CHCH_3$ E		t_{Bu}	0.30 (SS 16)	58.98 (58.86	7.38 7.41	6.08 6.06)
69	$-CH_2OCH_2CH=CHCH_3$ E	$(CH_3)_3CCO_2CH_2-$	t_{Bu}	0.57 (SS 16)	60.69 (60.41	8.15 8.26	5.89 6.04)
70	$-CH_2OCH_2CH=CHCH_3$ E		t_{Bu}	0.51, 0.62 (SS 16)	62.36 (62.46	8.20 8.47	5.58 5.60)
71	$-CH_2OCH_2CH=CHCH_3$ E		t_{Bu}	0.43, 0.51 (SS 16)	62.35 (62.53	7.64 7.60	5.71 5.76)

a. 0.0625 CH_2Cl_2
b. 0.22 CH_2Cl_2
c. 0.3 CH_2Cl_2

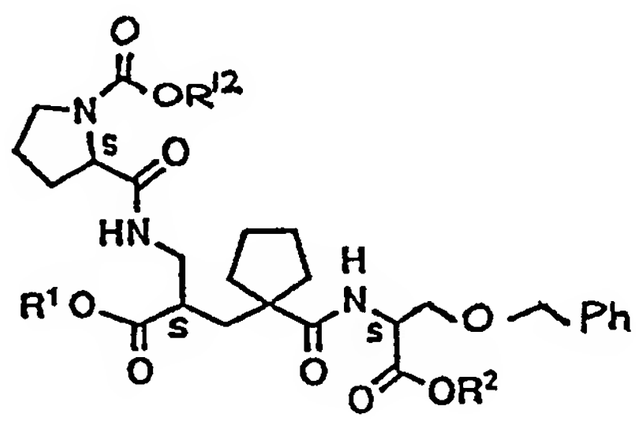
EXAMPLE 72

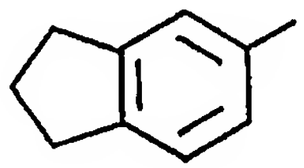
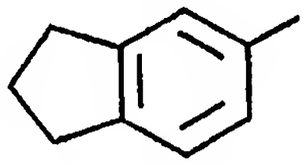
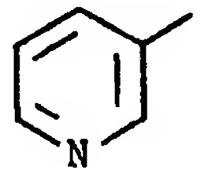
N-[1-(3-[N-(2,2,2-Trichloroethoxycarbonyl)-(S)-prolylamino]-2-(S)-(1-isobutyryloxy)ethoxycarbonyl)-1-cyclopentanecarbonyl]-O-benzyl-(S)-serine amide

Deprotection of Example 45 with trifluoroacetic acid according to Method A (Example 76), followed by conversion of the monocarboxylic acid product to its caesium salt and reaction with (1-isobutyryloxy)ethyl chloride following the procedures described above, gave the title compound as a white foam, Rf 0.44, 0.51 (SS 8). Found: C,52.63; H,6.21; N,7.15. $C_{34}H_{47}N_4O_{10}Cl_3$ requires C,52.48; H,6.09; N,7.20%.

EXAMPLES 73-75

The following esters were prepared from the appropriate acid and amine starting materials using the carbodiimide coupling described in Example 1.



Example No.	R^1	OR^2	R^{12}	Rf	Analysis % C H N (theoretical in brackets)
73		OEt	$PhCH_2$-	0.41 (SS 16)	68.85 6.99 5.44 (68.81 6.96 5.47)
74		NH_2	Cl_3CCH_2	0.60 (SS 8)	56.93 5.64 7.04 (56.89 5.94 7.17)
75		OEt	Cl_3CCH_2	0.28 (SS 8)	54.69 5.45 6.97 (54.59 5.63 7.27)

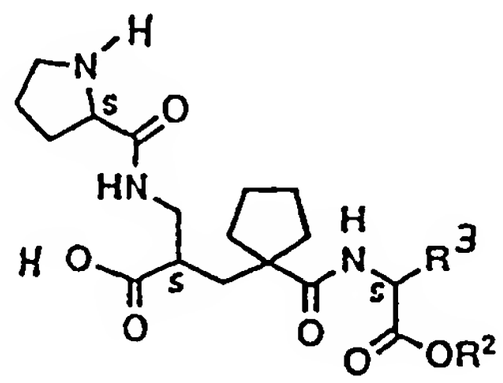
EXAMPLE 76 (DEPROTECTION METHOD A)

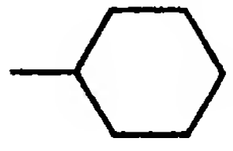
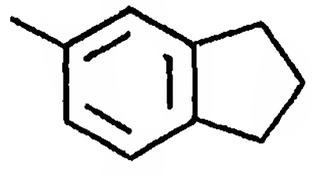
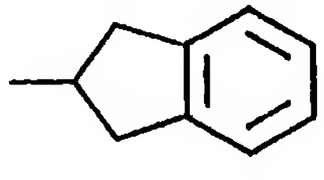
N-(1-[2(S)-Carboxy-3(S)-prolylaminopropyl]cyclopentanecarbonyl)-4-hydroxymethyl-(S)-phenylalanine-(3-phenyl)propyl ester

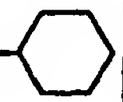
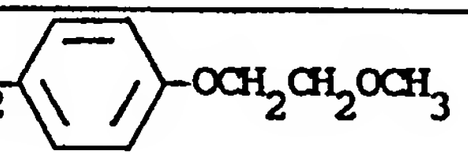
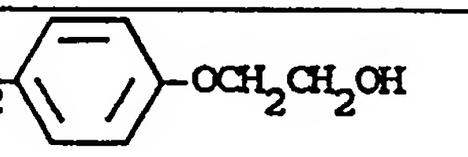
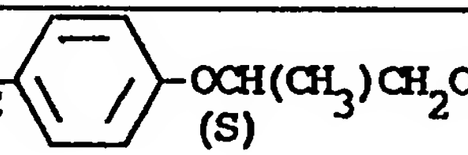
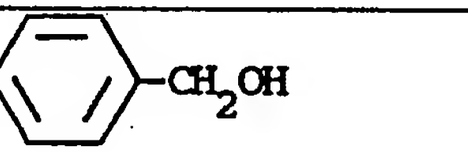
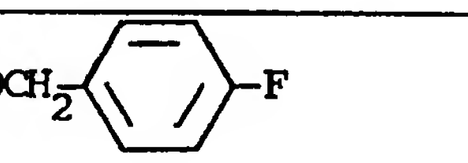
Trifluoroacetic acid (6 ml, 78 mmol) was added to a stirred, ice-cold solution of Example 13 (458 mg, 0.6 mmol) and anisole (973 mg, 9 mmol) in dichloromethane (6 ml). After 14 hours at 0°C, the reaction mixture was evaporated under vacuum and the residue azeotroped with toluene (3 x 20 ml) then dissolved in water (5 ml). The aqueous solution was washed with diethyl ether (2 x 50 ml), then subjected to ion-exchange chromatography (AG50W-X8 resin) using water and then 8% aqueous pyridine as eluents. Evaporation under vacuum of the appropriate (ninhydrin positive) fractions afforded the title compound as a white powder (210 mg, 55%), Rf 0.40 (SS 10). Found: C,66.26; H,7.46; N,6.85. $C_{34}H_{45}N_3O_7$; 0.50 H_2O requires C,66.21; H,7.52; N,6.81%.

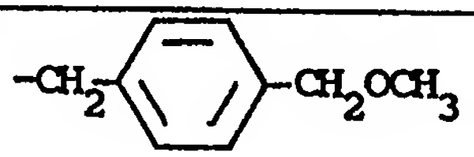
EXAMPLES 77-104

The following Examples were obtained from the corresponding diester using the appropriate deprotection Method A, (trifluoro-acetic acid, see above), B, (hydrogen chloride, described under Preparation 36), C, (catalytic hydrogenation, described under Preparation 37), D (formic acid, described under Preparation 24) or E (zinc in acetic acid, described under Preparation 66) to give the monoester products.



Example No.	Method	R^3	R^2	Rf	Analysis % C (theoretical in brackets)	H	N
77	B	$-CH_2OCH_2Ph$	$-CH_3$	0.30 (SS 10)			
78	B	$-CH_2OCH_2Ph$	$-CH(CH_2CH_3)_2$	0.31 (SS 10)	62.31 (62.36	8.07 8.19	7.20 7.27)a
79	B	$-CH(CH_3)OCH_2Ph$ (R)	$-CH_3$	0.28 (SS 10)	56.12 (55.80	7.03 7.45	6.95 7.23)b
80	B	$-CH_2OCH_2Ph$		0.37 (SS 10)	60.54 (60.50	7.75 7.67	6.72 6.83)c
81	B	$-CH_2OCH_2Ph$		0.58 (SS 10)	62.24 (62.27	6.98 6.99	6.29 6.40)d
82	B	$-CH_2OCH_2Ph$		0.30 (SS 10)	64.67 (64.53	7.35 7.33	6.52 6.64)e

83	B	$-CH_2OCH_2Ph$	$-CH_2CH_2CH_2$- 	0.26 (SS 10)	65.44 (65.56	8.10 8.42	6.55 6.75) f
84	B	$-CH_2OCH_2Ph$	$-(CH_2)_3Ph$	0.31 (SS 10)	61.81 (61.81	7.18 7.04	6.15 6.31) g
85	A	$-CH_2$-  $-OCH_2CH_2OCH_3$	H	0.15 (SS 10)	60.08 (59.65	7.28 7.23	7.66 7.73)
86	C then A	$-CH_2$-  $-OCH_2CH_2OH$	H	0.18 (SS 9)	58.13 (58.09	7.27 7.31	7.58 7.82) a
87	C then A	$-CH_2$-  $-OCH(CH_3)CH_2OH$ (S)	H	0.24 (SS 9)	59.57 (59.76	7.28 7.43	7.60 7.74)
88	A	$-CH_2$-  $-CH_2OH$	H	0.18 (SS 10)	61.19 (61.33	7.14 7.21	8.69 8.58)
89	B	$-CH_2OCH_2$-  $-F$	$-(CH_2)_3Ph$	0.19 (SS 9)	61.60 (61.67	6.62 6.85	6.09 6.35) h

90	A	$-CH_2OCH_2\underset{Cl}{\underset{\vert}{C}}=CH_2$	$-(CH_2)_3Ph$	0.20 (SS 9)	50.66 (51.11	5.57 5.67	5.18 5.39)i
91	B	$-CH_2CH_2OCH_2Ph$	$-(CH_2)_3Ph$	0.25 (SS 9)	63.34 (63.43	7.44 7.38	6.11 6.34)j
92	A		H	0.25 (SS 9)	62.04 (62.01	7.57 7.41	8.05 8.34)
93	A	$-CH_2OCH_2\underset{E}{CH=CH}CH_2OCH_3$	$-(CH_2)_3Ph$	0.21 (SS 22)	59.63 (59.38	7.33 7.48	6.32 6.49)k
94	A	$-CH_2OCH_2\underset{E}{CH=CH}CH_3$	$-(CH_2)_3Ph$	0.15 (SS 22)	60.65 (60.33	7.62 7.67	6.63 6.81)k
95	B	$-CH_2OCH_2CH=CH_2$	$-CH_2CH_3$	0.29 (SS 22)			
96	B	$-CH_2O(CH_2)_3CH_3$	$-CH_2Ph$	0.53 (SS 23)	59.90 (59.83	7.46 7.62	7.06 7.22)

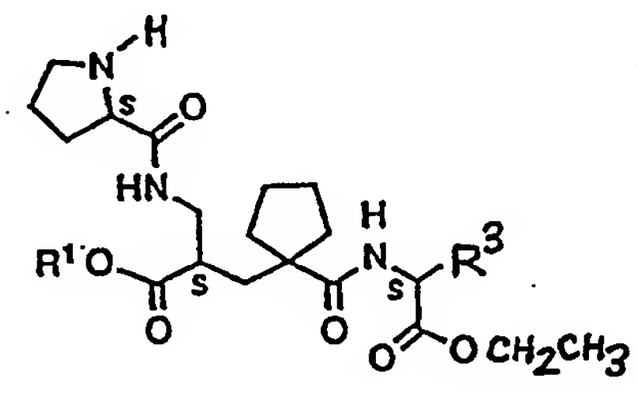
97	B	$-CH_2CH_2O(CH_2)_2CH_3$	$-CH_2CH_3$	0.45 (SS 10)		
98	B	$-CH_2O(CH_2)_2CH_3$	$-CH_2CH_3$	0.29 (SS 29)		
99	B	$-CH_2CH=CHCH_2OCH_2CH_3$ (E)	$-CH_3$	0.15 (SS 22)		
100	A	$-CH_2OCH_2C\equiv CCH_3$	$-CH_2Ph$	0.40 (SS 23)	62.08 (64.30	7.12 7.26	7.66 7.76)
101	A	$-CH_2O(CH_2)_3OCH_3$	$-(CH_2)_3Ph$	0.24 (SS 22)	59.55 (59.46	7.66 7.73	6.60 6.71)h
102	B	$-CH_2OCH_2C(=CH_2)CH_2OCH_3$	$-(CH_2)_3Ph$	0.40 (SS 22)	54.28 (54.36	6.36 6.64	5.39 5.51)i

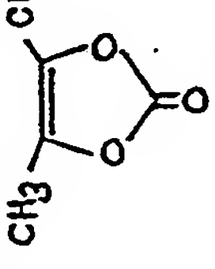
103	B	$-CH_2OCH_2C{\equiv}CH$	$-(CH_2)_3Ph$	0.05 (SS 22)	54.61 (54.63	6.28 6.26	5.75 5.93)g
104	E	$-CH_2OCH_2C(=CH_2)CH_3$	$-(CH_2)_3Ph$	0.09 (SS 22)	60.29 (60.45	7.93 7.77	6.40 6.61)m

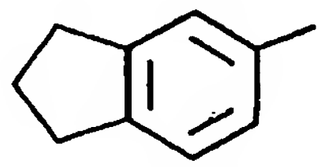
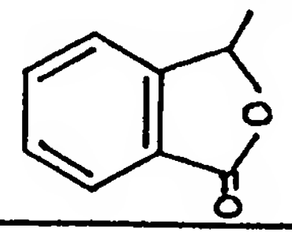
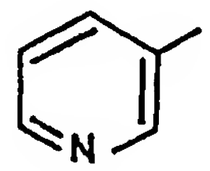
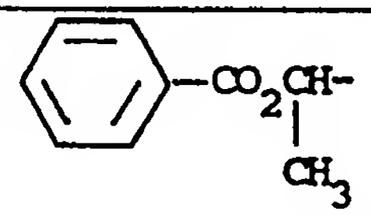
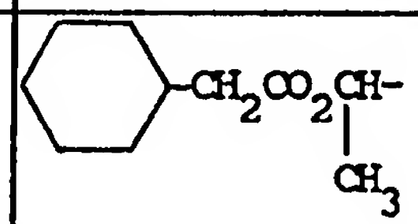
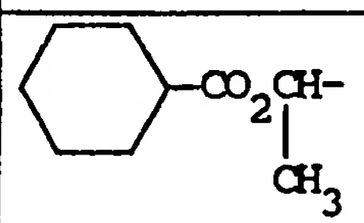
a. H_2O
b. HCl; 1.50 H_2O
c. HCl; 0.40 H_2O
d. HCl; 0.75 H_2O
e. 1.50 H_2O
f. 0.50 H_2O
g. HCl; 0.25 CH_2Cl_2
h. HCl
i. 1.5 CF_3CO_2H; 0.2 CH_2Cl_2
j. HCl; 0.25 H_2O
k. HCl; 0.5 H_2O
l. 1.25 CF_3CO_2H; H_2O
m. H_2O; HCO_2H

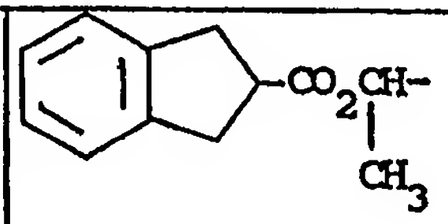
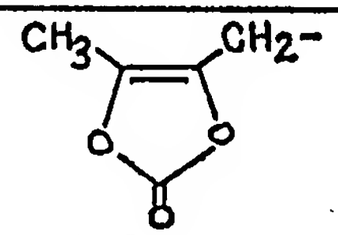
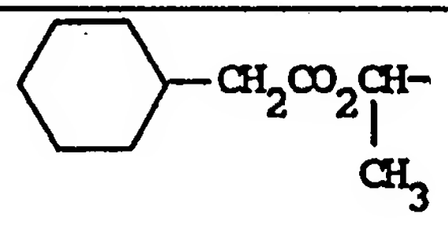
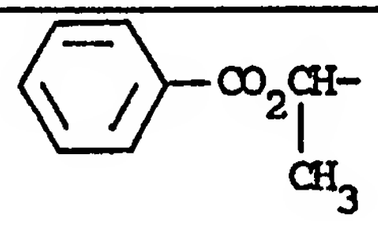
EXAMPLES 105-123

The following compounds were prepared by deprotection of the corresponding N-protected proline diester compound to give the following diester products. N-Trichloroethoxycarbonyl derivatives were deprotected following deprotection method E (zinc in acetic acid) and N-t-butyloxycarbonyl derivative were deprotected using deprotection method B (hydrogen chloride).



Example No.	Method	R^3	R^1	Rf		Analysis % C (theoretical in brackets) H N	
105	E	$-CH_2OCH_2Ph$	$(CH_3)_2CHCO_2CH(CH_3)-$	0.45 (SS 10)			
106	E	$-CH_2OCH_2Ph$	$CH_3(CH_2)_2CO_2CH(CH_3)-$	0.43 (SS 10)			
107	E	$-CH_2OCH_2Ph$	$CH_3CH_2OCO_2CH(CH_3)-$	0.40 (SS 10)	57.92 (57.35	7.45 6.35 7.22 6.26)a	
108	E	$-CH_2OCH_2Ph$	CH_3COCH_2-	0.34 (SS 10)	57.14 (57.08	7.30 6.30 7.05 6.58)b	
109	B	$-CH_2OCH_2Ph$		0.29 (SS 10)	56.61 (56.77	6.43 6.06 6.73 6.20)c	
110	E	$-CH_2OCH_2Ph$		0.28 (SS 22)			

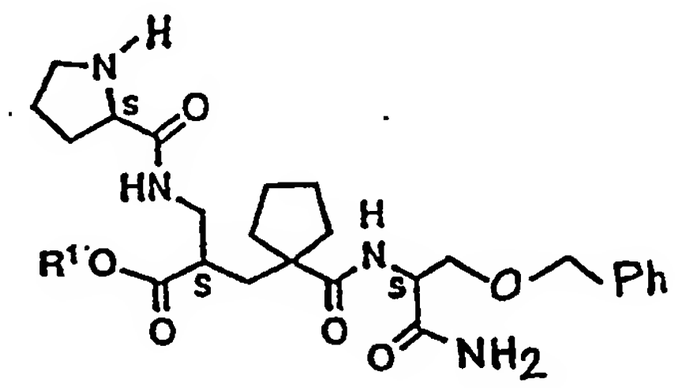
111	E	$-CH_2OCH_2Ph$		0.32 (SS 10)			
112	E	$-CH_2OCH_2Ph$		0.37 (SS 10)			
113	E	$-CH_2OCH_2Ph$		0.26 (SS 10)			
114	B	$-CH_2OCH_2CH{=}CHCH_2OCH_3$ E	$(CH_3)_3COO_2CH_2-$	0.30 (SS 22)	55.58 (55.47	7.90 7.96	6.05 6.26)d
115	B	$-CH_2OCH_2CH{=}CHCH_2OCH_3$ E		0.30 (SS 22)	57.91 (57.90	7.11 7.29	5.61 5.96)d
116	B	$-CH_2OCH_2CH{=}CHCH_2OCH_3$ E		0.30 (SS 22)	58.40 (58.69	8.04 8.16	5.78 5.89)a
117	B	$-CH_2OCH_2CH{=}CHCH_3$ E		0.30 (SS 22)	58.72 (58.33	7.28 8.13	6.05 6.18)e

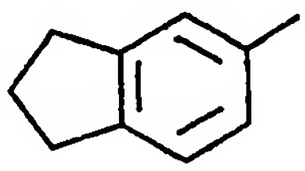
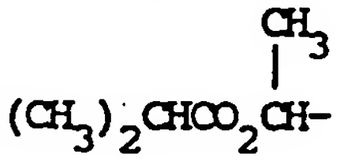
118	B	$-CH_2OCH_2CH=CHCH_3$ (E)		0.30 (SS 22)	55.97 (56.17	6.38 6.88	4.93 5.31) f
119	B	$-CH_2OCH_2CH=CHCH_3$ (E)	^nBu	0.25 (SS 22)	58.48 (58.57	8.18 8.43	7.18 7.32) a
120	B	$-CH_2OCH_2CH=CHCH_3$ (E)		0.20 (SS 22)	54.65 (54.50	6.95 7.10	6.39 6.57) d
121	B	$-CH_2OCH_2CH=CHCH_3$ (E)	$(CH_3)_3CCO_2CH_2-$	0.35 (SS 22)	56.88 (57.00	7.71 7.97	6.49 6.65) a
122	B	$-CH_2OCH_2CH=CHCH_3$ (E)		0.39 (SS 22)	59.22 (59.50	8.12 8.22	5.91 6.12) a
123	B	$-CH_2OCH_2CH=CHCH_3$ (E)		0.36 (SS 22)	59.29 (59.49	7.14 7.26	6.07 6.31) a

a. HCl
b. HCl; 0.33 CH_2Cl_2
c. HCl; 0.6 H_2O
d. HCl; 0.5 H_2O
e. HCl; 0.4 H_2O
f. HCl; CH_2Cl_2

EXAMPLES 124-126

These amides were obtained directly, in the case of Examples 124 and 125, by N-deprotection of the corresponding trichloroethoxycarbonyl proline precursors (Examples 74 and 72 respectively) using zinc and acetic acid (Method E). Example 126 was obtained from Example 45 in two stages, whereby Method E was followed by trifluoroacetic acid deprotection of the t-butyl ester (Method A).



Example No.	R^1	Rf	Analysis % (Theoretical in brackets) C	H	N
124		0.56 (SS 23)	60.85 (60.60	6.93 6.78	7.38 8.19)a
125		0.53 (SS 23)	47.85 (48.45	5.90 6.56	7.06 7.29)b
126	H	0.38 (SS 23)	58.29 (58.24	7.48 7.62	10.66 10.87)c

a. 0.5 CH_2Cl_2
b. 0.75 $ZnCl_2$; $2H_2O$
c. 1.5 H_2O

EXAMPLE 127

N-[1-{2(S)-Carboxy-3-[4(R)-hydroxy-(S)-prolylamino]propyl}cyclopentanecarbonyl]-O-benzyl-(S)-serine (3-phenyl)propyl ester

This was obtained from Example 48 by reaction with hydrogen chloride in dichloromethane (deprotection Method B) to furnish the title compound, Rf 0.45 (SS 10). Found: C,60.78; H,6.88; N,6.22. $C_{34}H_{45}N_3O_{10}$; HCl; H_2O requires C,60.21; H,7.13; N,6.20%.

EXAMPLE 128

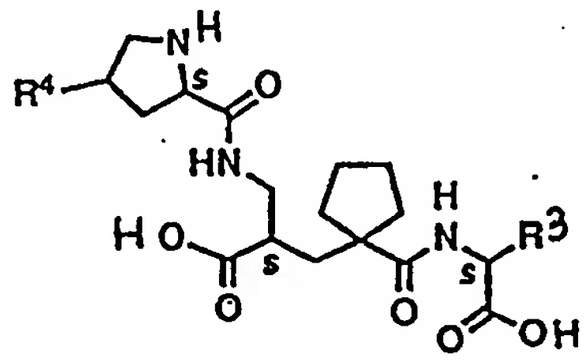
N-{1[2(S)-Carboxy-3-(S)-prolylaminopropyl]cyclopentanecarbonyl}-O-benzyl-(S)-serine

1M Aqueous sodium hydroxide solution (1.5 ml, 1.5 mmol) was added to a stirred solution of Example 77 (171 mg, 0.30 mmol) in 1,4-dioxan (2 ml). After 48 hours the pH of the solution was adjusted to 8 using dilute hydrochloric acid, then the solution subjected to ion-exchange chromatography (AG50 resin) using water and then a 1-10% aqueous pyridine gradient as eluents. Evaporation under vacuum of the appropriate (ninhydrin positive) fractions, followed by freeze drying of an aqueous solution of the residue, furnished the title compound as a white powder (108 mg, 70%), Rf (SS 10). Found: C,59.52; H,7.19; N,8.28. $C_{25}H_{35}N_3O_7$; H_2O requires C,59.15; H,7.35; N,8.28%.

EXAMPLES 129-148

The following Examples were obtained by hydrolysis of the appropriate ester following the procedure of Example 128.

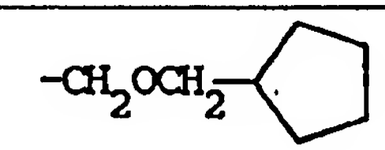
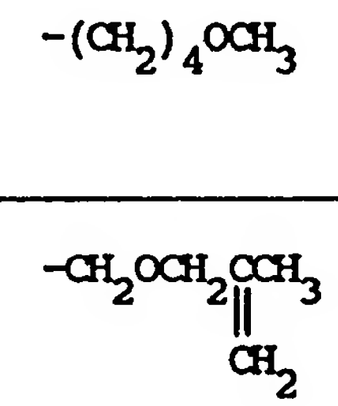
Examples 129-147 are unsubstituted (S)-proline derivatives (R^4 = H) while Example 148 is the 4(R)-hydroxy-(S)-proline derivative (R^4 = OH) derived from Example 127.



Example No.	R^3	Rf	Analysis % C (theoretical in brackets)	H	N
129	$-CH(CH_3)OCH_2Ph$ (R)	0.16 (SS 10)	58.99 (58.84	7.48 7.60	7.61 7.91)a
130	$-CH_2OCH_2$-(4-F-phenyl)	0.06 (SS 9)	57.51 (57.73	6.63 6.86	8.09 8.08)
131	$-CH_2OCH_2C(Cl)=CH_2$	0.20 (SS 10)	53.11 (53.22	6.90 6.80	8.87 8.87)
132	$-CH_2CH_2OCH_2Ph$	0.20 (SS 10)	60.79 (60.92	7.66 7.47	8.20 8.20)

Example No.	R^3	Rf	Analysis % C (theoretical in brackets)	H	N
133	$-CH_2OCH_2CH=CHCH_2OCH_3$ (E)	0.08 (SS 22)	56.18 (56.08	7.60 7.78	8.52 8.53)b
134	$-CH_2OCH_2CH=CHCH_3$ (E)	0.03 (SS 22)	57.27 (57.13	7.49 7.84	9.31 9.08)b
135	$-CH_2OCH_2CH=CH_2$	0.08 (SS 22)	56.62 (56.92	8.05 7.59	9.37 9.48)c
136	$-CH_2O(CH_2)_3CH_3$	0.27 (SS 10)	52.47 (51.80	7.62 7.90	8.07 8.24)d
137	$-CH_2CH_2O(CH_2)_2CH_3$	0.27 (SS 10)	57.48 (57.54	8.13 8.20	9.15 9.15)

138	$-CH_2O(CH_2)_2CH_3$	0.09 (SS 22)	55.93 (55.53	7.67 7.78	9.13 9.16)e
139	$-CH_2\underset{E}{CH=CH}CH_2OCH_2CH_3$	0.07 (SS 22)	59.14 (59.08	7.56 7.98	8.81 8.99)
140	$-CH_2OCH_2C{\equiv}CCH_3$	0.23 (SS 10)	56.13 (56.27	7.37 7.51	8.86 8.95)d
141	$-CH_2O(CH_2)_3OCH_3$	0.07 (SS 22)	55.70 (56.03	7.77 7.91	8.65 8.91)
142	$-CH_2OCH_2\underset{\|}{\overset{CH_2}{C}}CH_2OCH_3$	0.03 (SS 22)	55.98 (56.08	7.76 7.78	8.50 8.53)b
143	$-CH_2OCH_2C{\equiv}CH$	0.08 (SS 22)	57.11 (57.06	7.13 7.18	9.37 9.51)f

144	$-CH_2O(CH_2)_2CH(CH_3)_2$	0.10 (SS 22)	59.13 (58.83	8.21 8.37	8.90 8.95)
145		0.09 (SS 22)	59.44 (59.86	8.27 8.16	8.58 8.73)
146	$-(CH_2)_4OCH_3$	0.10 (SS 22)	57.81 (58.00	8.05 8.19	9.28 9.22)
147		0.26 (SS 22)	57.15 (57.13	7.69 7.85	9.16 9.09)a
148	$-CH_2OCH_2Ph$	0.24 (SS 10)	54.67 (54.64	6.96 7.07	8.16 7.96)g

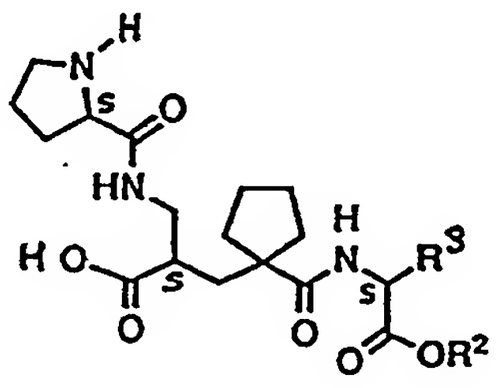
a. 1.5 H_2O
b. 0.5 H_2O
c. 0.2 H_2O
d. H_2O
e. 0.2 CH_2Cl_2
f. 0.25 H_2O
g. $2H_2O$

EXAMPLES 149-158

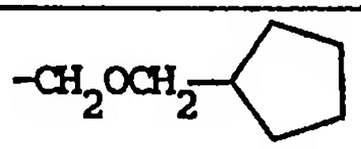
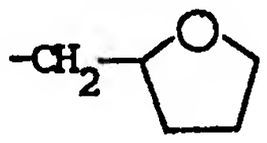
Examples 149-153 were obtained from the appropriate precursor N-protected proline diester derivatives (Examples 29-33 respectively) by catalytic hydrogenation (deprotection Method C) followed by treatment of the resulting monoacids with hydrogen chloride (deprotection Method B).

Examples 154-156 were obtained from similar precursors (Examples 36, 35 and 40 respectively) using hydrogen chloride treatment only (deprotection Method B).

Examples 157-158 are the result of three successive deprotection steps from their analogous precursors (Examples 41 and 42 respectively): base hydrolysis of the (3-phenyl)propyl ester group according to the method of Example 128, but using routine extraction procedures rather than ion-exchange cnromatography to isolate the monoacid, followed by Method B and finally Method C.



Example No.	R^3	R^2	Rf	Analysis % C	H	N (theoretical in brackets)
149	$-CH_2OCH_2$-cyclopropyl	H	0.13 (SS 24)			
150	$-CH_2OCH_2CF_3$	H	0.13 (SS 24)			
151	$-CH_2OCH(CH_3)_2$	H	0.13 (SS 24)	51.85 (51.79	7.78 7.66	8.03 8.63)a
152	$-CH_2OCH(CH_3)CF_3$	H	0.13 (SS 24)	47.08 (47.41	6.33 6.25	7.35 7.90)b
153	$-CH_2O$-cyclopentyl	H	0.13 (SS 24)	53.88 (53.84	7.63 7.66	7.80 8.19)a

154	$-CH_2O(CH_2)_2CH(CH_3)_2$	$-CH_2CH_3$	0.26 (SS 22)	
155		$-CH_2CH_3$	0.26 (SS 22)	
156	$-(CH_2)_4OCH_3$	CH_3	0.18 (SS 22)	
157	$-CH_2OCH_3$	H	0.10 (SS 10)	
158		H	0.13 (SS 10)	

a. HCl, 0.5 H_2O
b. HCl

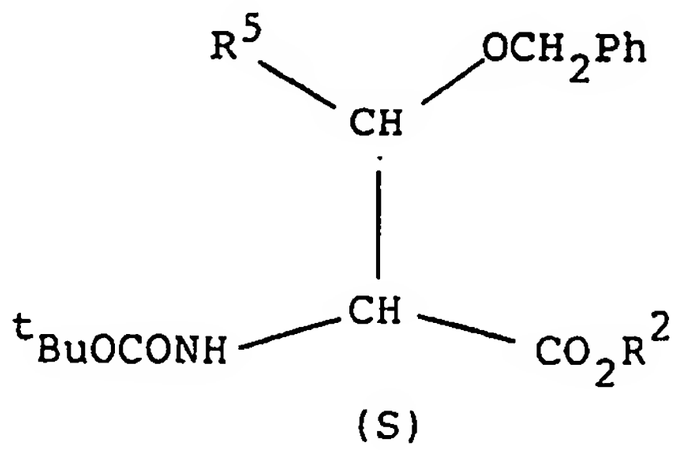
PREPARATION 1

N-t-Butoxycarbonyl-O-benzyl-(S)-serine methyl ester

N-t-Butoxycarbonyl-O-benzyl-(S)-serine (2.34 g, 7.9 mmol) was added to a stirred suspension of anhydrous potassium carbonate (2.19 g, 15.8 mmol) in dimethylformamide (25 ml), followed by iodomethane (1.34 g, 9.4 mmol). After 48 hours at room temperature, the reaction mixture was evaporated under vacuum and the residue partitioned between ethyl acetate (100 ml) and 2M hydrochloric acid (50 ml). The organic phase was separated, washed successively with 2M hydrochloric acid (50 ml), saturated aqueous sodium bicarbonate solution (50 ml) and saturated brine (50 ml), then dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave the required product as a colourless oil (2.40 g, 97%), Rf 0.57 (silica; SS 1). Found: C,62.18; H,7.48; N,4.63. $C_{16}H_{23}NO_5$ requires C,62.11; H,7.49; N,4.53%.

PREPARATIONS 2-5

The following Preparations were effected according to the procedure of Preparation 1 using the appropriate α-amino acid derivative and alkyl halide.



Preparation No.	R^2	R^5	Rf	Analysis % C H N (theoretical in brackets)
2	$-CH_2CH_2CH_2Ph$	H	0.65 (SS 2)	69.68 7.48 3.55 (69.70 7.56 3.39)
3	$-CH(CH_2CH_3)_2$	H	0.62 (SS 3)	
4	$-CH_3$	(R) CH_3	0.59 (SS 3)	
5	$-CH_2CH_3$	H	0.60 (SS 3)	62.45 7.79 4.10 (62.27 7.84 4.27)a

a. 0.25 H_2O

PREPARATION 6

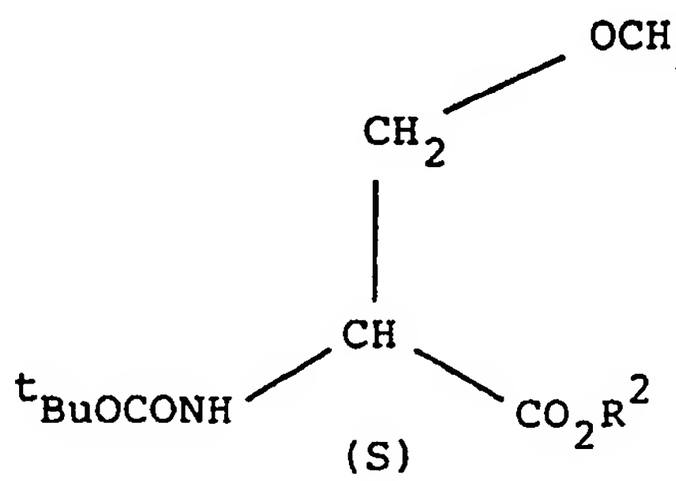
N-t-Butoxycarbonyl-O-benzyl-(S)-serine cyclohexyl ester

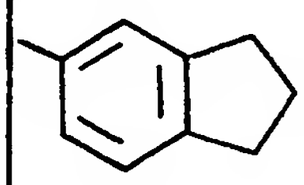
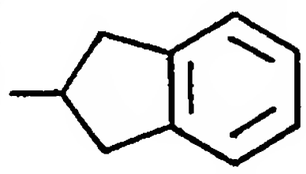
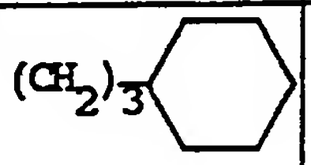
To a stirred solution of N-t-butoxycarbonyl-O-benzyl-(S)-serine (5.00 g, 16.9 mmol) and cyclohexanol (3.39 g, 33.8 mmol) in dichloromethane (50 ml) at 0°C were added, sequentially, 1-hydroxybenzotriazole (2.75 g, 20.3 mmol), N-methylmorpholine (4.27 g, 42.3 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (4.22 g, 22.0 mmol) and 4-dimethylaminopyridine (0.21 g, 1.7 mmol). The ice bath was removed, then the reaction mixture was stirred for 24 hours at room temperature and evaporated under vacuum. The residue was partitioned between ethyl acetate (200 ml) and 2M hydrochloric acid (100 ml), then the

organic phase was separated, washed successively with 2M hydrochloric acid (2 x 100 ml), saturated aqueous sodium bicarbonate solution (2 x 100 ml) and saturated brine (100 ml), dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate provided an oil (5.47 g), which was purified chromatographically using silica gel (200 g) and an 8:2 mixture of hexane:ether as eluent. Evaporation under vacuum of the appropriate fractions afforded the title compound as a colourless oil (1.74 g, 27%), Rf 0.62 (silica; SS 2), $[\alpha]_D^{25}$ -63.0° (c = 0.1, MeOH). Found: C,66.61; H,8.34; N,3.71. $C_{21}H_{31}NO_5$ requires C,66.81; H,8.28; N,3.71%.

PREPARATIONS 7-9

The following Preparations were effected according to the procedure of Preparation 6 using N-t-butoxycarbonyl-O-benzyl-(S)-serine and the appropriate alcohol or phenol.



Preparation No.	R^2	Rf	Analysis % C H N (theoretical in brackets)
7			69.73 7.11 3.35 (70.05 7.10 3.40)
8		0.66 (SS 3)	69.70 7.16 3.49 (70.05 7.10 3.40)
9		0.53 (SS 2)	68.69 8.92 3.70 (68.70 8.89 3.34)

PREPARATION 10

N-t-Butoxycarbonyl-O-benzyl-(S)-serine amide

N-t-Butoxycarbonyl-O-benzyl-(S)-serine (10.0 g, 34 mmol) was added to a stirred suspension of ammonium bicarbonate (8.0 g, 102 mmol) and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (10.05 g, 41 mmol) in chloroform (80 ml). After 48 hours at room temperature the solvent was removed under vacuum and the residue partitioned between ethyl acetate (200 ml) and 2N hydrochloric acid (50 ml). The ethyl acetate phase was separated, washed with saturated sodium bicarbonate (50 ml) then brine (20 ml), dried ($MgSO_4$), then filtered. Evaporation under vacuum of the filtrate followed by crystallisation of the residue from ethyl acetate/hexane gave the title compound as a crystalline white solid. Found: C,61.38; H,7.35; N,9.48. $C_{15}H_{22}N_2O_4$ requires C,61.20; H,7.53; N,9.51%.

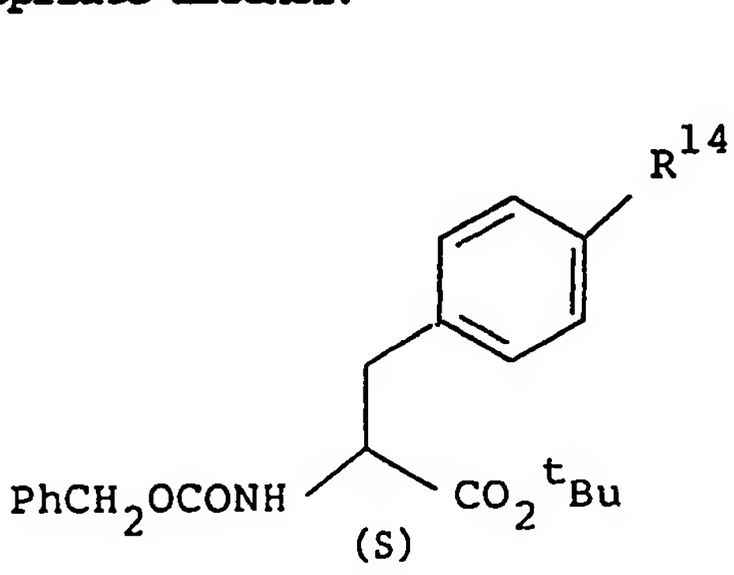
PREPARATION 11

N-Benzyloxycarbonyl-O-(2-methoxyethyl)-(S)-tyrosine t-butyl ester

Diethyl azodicarboxylate (4.4 ml, 28 mmol) was added under nitrogen to a stirred, ice-cold solution of N-benzyloxycarbonyl-(S)-tyrosine t-butyl ester (5.2 g, 14 mmol), triphenylphosphine (7.34 g, 28 mmol) and 2-methoxyethanol (3.3 ml, 42 mmol) in dry tetrahydrofuran (30 ml). The ice bath was removed, then the reaction mixture was stirred for 24 hours at room temperature and evaporated under vacuum. The residue was digested with a hot 1:1 mixture of ether and hexane, then the supernatant solution was chromatographed on silica gel (500 g) using a 1:1 mixture of ether and hexane as eluent. Evaporation under vacuum of the required fractions furnished the title compound as an oil (5.8 g, 95%), Rf 0.30 (silica; SS 2).

PREPARATIONS 12-13

The following Preparations were effected according to the procedure of Preparation 11 using N-benzyloxycarbonyl-(S)-tyrosine and the appropriate alcohol.



Preparation No.	R^{14}	Rf	Analysis % C H N (theoretical in brackets)
12	$-OCH_2CH_2OCH_2Ph$	0.25 (SS 4)	71.21 6.92 2.83 (71.27 6.98 2.77)
13	$-OCH(CH_3)CH_2OCH_2Ph$ (S)	0.42 (SS 2)	71.74 7.15 2.75 (71.65 7.18 2.70)

PREPARATION 14

N-Benzyloxycarbonyl-O-trifluoromethanesulphonyl-(S)-tyrosine t-butyl ester

To a stirred solution of N-benzyloxycarbonyl-(S)-tyrosine t-butyl ester (2.22 g, 5.97 mmol) in dichloromethane (20 ml) at -78°C were added, sequentially , triethylamine (1.21 g, 11.94 mmol) and N-phenyl trifluoromethanesulphonimide (2.13 g, 5.97 mmol). The cooling bath was removed, then the reaction mixture was stirred for 24 hours at room temperature and evaporated under vacuum. The residue was partitioned between ethyl acetate (200 ml) and saturated aqueous sodium bicarbonate solution (100 ml), then the organic phase separated, washed with saturated aqueous sodium bicarbonate solution (50 ml) and saturated brine (50 ml), dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave an oil, which was purified by chromatography on silica gel (100 g) using an elution gradient of hexane:ether: diethylamine (90:10:1 to 50:50:1). Evaporation under vacuum of the required fractions provided the title compound as a white

crystalline solid (2.45 g, 82%), m.p. 48-49°C, $[\alpha]_D^{25}$ -33° (c = 0.1, MeOH). Found: C,52.51; H,4.76; N,2.76. $C_{22}H_{24}F_3NO_7S$ requires C,52.47; H,4.80; N,2.78%.

PREPARATION 15

N-Benzyloxycarbonyl-4-hydroxymethyl-(S)-phenylalanine t-butyl ester

(i) N-Benzyloxycarbonyl-4-(2-ethoxycarbonyl-1-ethenyl-(S)-phenyl-alanine t-butyl ester

A solution of the product of Preparation 14 (12.99 g, 25.8 mmol), ethyl acrylate (5.6 ml, 51.6 mmol), triethylamine (16 ml) and bis(triphenylphosphine)palladium (II) chloride (1.05 g, 1.5 mmol) was stirred under nitrogen at 100-110°C for 18 hours. Evaporation under vacuum followed by dilution of the residue with water gave a suspension which was extracted with a 1:1 mixture of hexane and ether. The organic extract was washed successively with 1M hydrochloric acid, water, saturated aqueous sodium bicarbonate solution and saturated brine, then dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave a yellow oil (11.5 g) which was chromatographed on silica gel (600 g) using a 3:2 mixture of hexane and ether as eluent. Evaporation under vacuum of the appropriate fractions afforded the required olefin as a viscous oil (8.42 g, 72%), Rf 0.42 (SS 2). Found: C, 69.10; H,6.99; N,3.07. $C_{26}H_{31}NO_6$ requires C,68.86; H,6.89; N,3.09%.

(ii) N-Benzyloxycarbonyl-4-(2-ethoxycarbonyl-1,2-dihydroxyethyl)-(S)-phenylalanine t-butyl ester

To a stirred solution of the previous product (8.42 g, 18.56 mmol) in a mixture of acetone (30 ml) and water (8 ml) were added, sequentially, N-methylmorpholine-N-oxide (3.78 g, 28 mmol) and a 2.5% solution of osmium tetroxide in t-butanol (1.5 ml). After 18 hours acetone (50 ml) was added, the reaction mixture evaporated under vacuum then the residue chromatographed on silica gel (500 g) using a 4:1 mixture of ether and hexane as eluent. Evaporation under vacuum of the appropriate fractions furnished the required diol as a gum (8.62 g, 95%), Rf 0.25 (SS 5). Found: C,64.33; H,6.72; N,3.00. $C_{26}H_{33}NO_8$ requires C,64.05; H,6.82; N,2.87%.

(iii) N-Benzyloxycarbonyl-4-formyl-(S)-phenylalanine t-butyl ester

A solution of the previous product (8.61 g, 17.7 mmol) in ether (200 ml) was vigorously stirred with a solution of sodium periodate (7.55 g, 35.3 mmol) in water (150 ml) for 20 hours. The ether phase was separated, washed with water, dried ($MgSO_4$) and evaporated under vacuum to provide an oil (7.03 g) which, after chromatography on silica gel using a 1:1 mixture of hexane and ether as eluent, gave the required aldehyde as a clear oil (6.68 g, 99%), Rf 0.30 (SS 2). Found: C,68.80; H,6.54; N,3.68. $C_{22}H_{25}NO_5$ requires C,68.91; H,6.57; N,3.65%.

(iv) Title compound

Sodium borohydride (330 mg, 8.7 mmol) was added to a stirred, ice-cold solution of the previous product (6.67g, 17.4 mmol) in ethanol (60 ml). After 10 minutes the reaction solution was

acidified to pH 6 with 1M hydrochloric acid, then evaporated under vacuum. The residue was partitioned between ether and water, then the organic phase separated, washed successively with 1M hydrochloric acid, water, saturated aqueous sodium bicarbonate solution and saturated brine, dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate provided an oil which was chromatographed on silica gel using a 7:3 mixture of ether and hexane as eluent. Evaporation under vacuum of the appropriate fractions afforded the required alcohol as an oil (6.6 g, 98%), which solidified on chilling. Rf 0.33 (SS 6), $[\alpha]_D^{25}$ +47.8° (c = 1.04, CH_2Cl_2). Found: C,68.21; H,7.09; N,3.66. $C_{22}H_{27}NO_5$ requires C,68.55; H,7.06; N,3.63%.

PREPARATION 16

N-Benzyloxycarbonyl-4-hydroxymethyl-(S)-phenylalanine (3-phenyl)-propyl ester

The t-butyl ester group of the product of Preparation 15 was dealkylated by Method B described in Preparation 36, then the resulting acid was realkylated using (3-phenyl)propyl bromide according to the method of Example 54 to give the title compound as an oil, Rf 0.25 (SS 6). Found: C,72.35; H,6.42; N,3.12. $C_{27}H_{29}NO_5$ requires C,72.46; H,6.53; N,3.13%.

PREPARATION 17

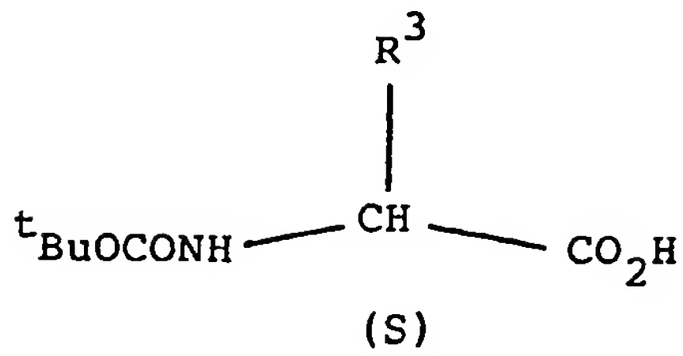
N-t-Butoxycarbonyl-O-(4-fluorobenzyl)-(S)-serine

Sodium hydride (80% dispersion in oil; 2.25 g, 75.05 mmol)

was added under nitrogen to a stirred solution of N-t-butoxy-carbonyl-(S)-serine (7.0 g, 34.11 mmol) in dry tetrahydrofuran (150 ml) at 0°C. The ice-bath was removed, stirring continued for 4 hours, then a solution of 4-fluorobenzyl chloride (5.42 g, 37.5 mmol) in dry tetrahydrofuran (50 ml) was added dropwise. After a further 15 hours more sodium hydride (0.51 g, 17.0 mmol) was added followed, 4 hours later, by more 4-fluorobenzyl chloride (2.47 g, 17.0 mmol). The reaction mixture was stirred for a further 20 hours and then, after destruction of excess sodium hydride using 2-propanol, evaporated under vacuum. The residue was dissolved in water (70 ml), then the solution washed with ether and acidified to pH 3.5 with 2M hydrochloric acid. Exhaustive extraction with ethyl acetate, washing of the combined extracts with water, followed by evaporation under vacuum of the dried ($MgSO_4$) extracts, provided an oil (6.3 g). Purification of the oil by chromatography on silica gel using an elution gradient of ethyl acetate:hexane (from 50:50 to 100:0), followed by elution with ethanol:ethyl acetate (10:90), furnished the required product as a colourless solid (1.95 g, 18%), Rf 0.40 (SS 7). Found: C,55.16; H,5.74; N,4.06. $C_{15}H_{20}FNOS$; 0.20 CH_2Cl_2 requires C,55.27; H,6.22; N,4.24%.

PREPARATIONS 18-22

The following Preparations were effected according to the procedure of Preparation 17 using the appropriate alkylating agents. For Preparations 19 and 20, N-t-butoxycarbonyl-(S)-homoserine was the starting material.



(S)

Preparation No.	R^3	Rf
18	$-CH_2OCH_2C(Cl)=CH_2$	0.30 (SS 8)
19	$-CH_2CH_2OCH_2Ph$	0.40 (SS 8)
20	$-(CH_2)_2O(CH_2)_2CH_3$	0.25 (SS 6)
21	$-CH_2OCH_2C\equiv CH$	0.3 (SS 7)
22	$-CH_2OCH_2C(=CH_2)-CH_3$	0.48 (SS 7)

PREPARATION 23

N-Triphenylmethyl-O-2-propenyl-(S)-serine ethyl ester

40% Aqueous sodium hydroxide solution (0.87 ml, 8.66 mmol) was added at 10°C to a stirred solution of N-triphenylmethyl-S-serine ethyl ester (2.5 g, 6.66 mmol), allyl bromide (886 mg, 7.32 mmol) and benzyltriethylammonium chloride (1.51 g, 6.66 mmol) in dichloromethane (10 ml). The mixture was allowed to warm to room temperature and stirred overnight. Dichloromethane was added, then the solution washed with water, dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave an oil which was chromatographed on silica, eluting with a 4:1 mixture of hexane and ethyl acetate, to give the pure product as a colourless oil (2.14 g, 77%), Rf 0.52 (SS 1), $[\alpha]_D^{25}$ + 52.3 (c = 0.99, CH_2Cl_2).

Found: C,76.94; H,7.10; N,3.25. $C_{27}H_{29}NO_3$; 0.1 CH_2Cl_2 requires C,76.76; H,6.94; N,3.30%.

PREPARATION 24 (DEPROTECTION METHOD D)

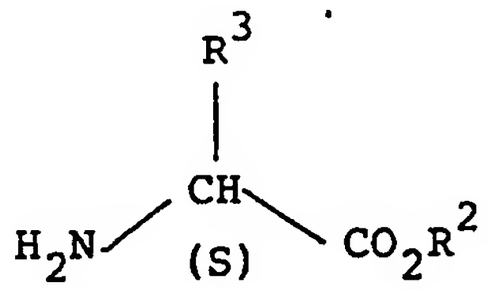
O-(2-Propenyl)-(S)-serine ethyl ester

A solution of the above product (1.0 g, 2.4 mmole) in formic acid (15 ml) was allowed to stand at room temperature for 5 hours. The solvent was then evaporated under vacuum and the residue dried azeotropically with acetonitrile. The resulting white solid was dissolved in water (15 ml) and the solution extracted with diethyl ether. The aqueous phase was basified to pH 9 with sodium carbonate, extracted with ethyl acetate (x 3) and dichloromethane (x 2), and then the combined organic extracts dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave the pure

product as an oil (319 mg, 76%), Rf 0.29 (SS 21). Found: C,55.98; H,8.80; N7.99. $C_8H_{15}NO_3$ requires C,55.47; H,8.73; N,8.09%.

PREPARATIONS 25-29

The following compounds were prepared from the corresponding N-trityl-(S)-serine ester by alkylation under phase transfer conditions followed by formic acid deprotection as described in the above Preparations. Preparations 25 to 28 are (3-phenyl)propyl esters (R^2=$(CH_2)_3Ph$), Preparation 29 is the ethyl ester ($R^2 = CH_2CH_3$).

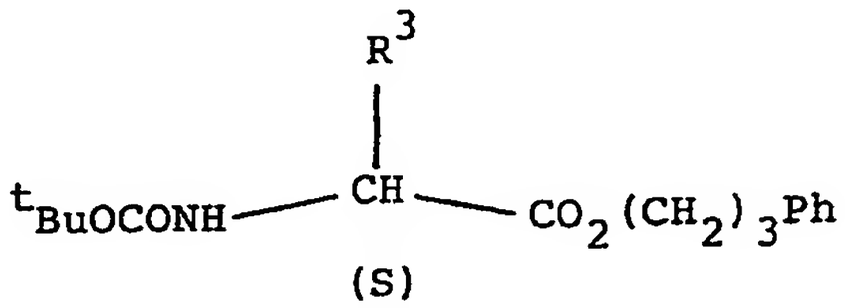


Example No.	R^3	Rf	Analysis % (Theoretical in brackets) C	H	N
25	$-CH_2OCH_2CH{=}CHCH_2OCH_3$ E	0.54 (SS 22)	66.06 (66.42	7.89 8.20	4.47 4.56)
26	$-CH_2OCH_2CH{=}CHCH_3$ E	0.45 (SS 22)	61.34 (61.24	7.80 7.71	4.46 4.46)a
27	$-CH_2O(CH_2)_3OCH_3$	0.42 (SS 22)	64.84 (65.06	8.49 8.53	4.80 4.74)
28	$-CH_2OCH_2C(=CH_2)CH_2OCH_3$	0.35 (SS 8)	66.39 (66.43	7.92 8.20	4.57 4.56)
29	$-CH_2O(CH_2)_2CH(CH_3)_2$	0.35 (SS 22)			

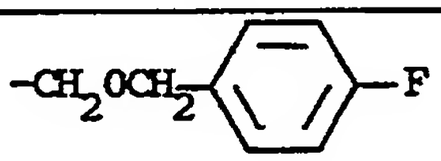
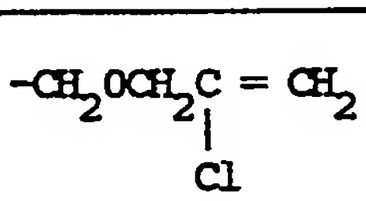
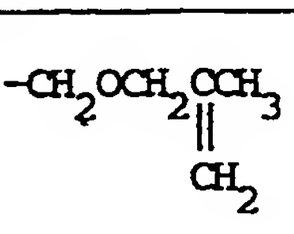
a. HCl

PREPARATIONS 30-35

The following Preparations were effected according to the procedure of Example 54 using (3-phenyl)propyl bromide with the respective products of Preparations 17-22.



(S)

Prep. No.	R^3	Rf	Analysis % C (theoretical in brackets)	H	N
30		0.35 (SS 1)	66.64 (66.80	7.09 7.01	3.23 3.25)
31		0.30 (SS 1)			
32	$-(CH_2)_2OCH_2Ph$	0.40 (SS 1)			
33	$-(CH_2)_2O(CH_2)_2CH_3$	0.25 (SS 6)	58.07 (58.11	9.26 9.40	4.89 4.84)
34	$-CH_2OCH_2C{\equiv}CH$	0.38 (SS 1)	66.56 (66.46	7.56 7.53	3.92 3.83)
35		0.51 (SS 1)	66.50 (66.82	8.30 8.28	3.81 3.71)

PREPARATION 36 (DEPROTECTION METHOD B)

O-Benzyl-(S)-serine methyl ester hydrochloride

A stirred, ice-cold solution of the product of Preparation 1 (2.05g, 66.3 mmol) in dichloromethane (50 ml) was saturated with hydrogen chloride. After a further 3 hours at 0°C, the reaction mixture was evaporated under vacuum and the residual solid azeotroped with dichloromethane (3 x 30 ml) to afford the title compound as a white powder (1.67 g, 97%), Rf 0.60 (SS 9), $[\alpha]_D^{25}$ +21° (c = 0.1, MeOH). Found: C,54.14; H,6.54; N,5.75. $C_{11}H_{15}NO_3$; HCl requires C,53.77; H,6.56; N,5.70%.

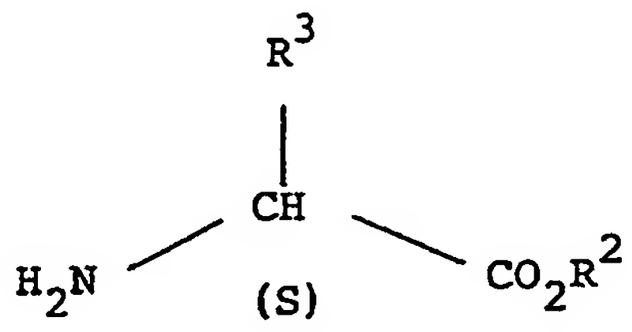
PREPARATION 37 (DEPROTECTION METHOD C)

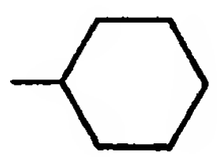
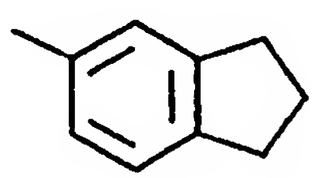
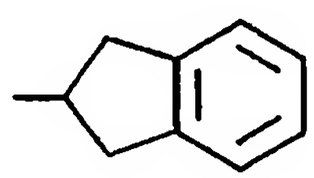
O-(2-Methoxyethyl)-(S)-tyrosine t-butyl ester

A solution of the product of Preparation 11 (5.4 g) in a mixture of ethanol (40 ml) and water (10 ml) was hydrogenated over 5% palladium on charcoal at 50 psi (3.45 bar) for 2 hours. The reaction mixture was filtered, the filtrate evaporated under vacuum and the residual oil chromatographed on silica gel (300 g) using a 1:39 mixture of ethanol and ethyl acetate as eluent.. Evaporation under vacuum of the required fractions afforded the title compound as a clear oil (3.07 g, 82%), Rf 0.30 (SS 8), $[\alpha]_D^{25}$ + 10.1° (c = 1.63, CH_2Cl_2). Found: C,64.67; H,8.54; N,4.63. $C_{16}H_{25}NO_4$ requires C,65.06; H,8.53; N,4.74%.

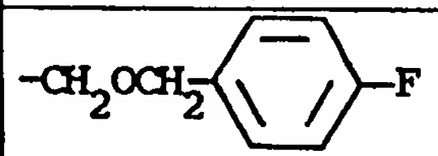
PREPARATIONS 38-55

The following Preparations were effected using deprotection Methods A, B or C, with the corresponding N-protected amino acid ester starting material. Method A is described under Example 76 (trifluoroacetic acid), Method B is described under Preparation 36 (hydrogen chloride) and Method C is described under Preparation 37 (hydrogenation).



Preparation No.	Method	R^3	R^2	Rf	Analysis % C (theoretical in brackets)	H	N
38	B	$-CH_2OCH_2Ph$	$CH_2CH_2CH_2Ph$	0.83 (SS 9)	64.59 (64.89	6.95 6.95	3.95 3.98) a
39	B	$-CH_2OCH_2Ph$	$CH(CH_2CH_3)_2$	0.59 (SS 10)	59.89 (59.69	7.90 8.01	4.49 4.64) b
40	B	$-CH(CH_3)OCH_2Ph$ (R)	CH_3	0.43 (SS 10)			
41	B	$-CH_2OCH_2Ph$		0.55 (SS 10)	61.10 (61.23	7.75 7.71	4.39 4.46) b
42	B	$-CH_2OCH_2Ph$			62.70 (62.64	6.70 6.14	3.79 3.79) c
43	B	$-CH_2OCH_2Ph$		0.50 (SS 10)	65.45 (65.60	6.49 6.37	4.04 4.03) b

Preparation No.	Method	R^3	R^2	Rf	Analysis % C H N (theoretical in brackets)
44	B	$-CH_2OCH_2Ph$	$-CH_2CH_2CH_2$-(cyclohexyl)	0.57 (SS 10)	63.46 8.31 4.33 (63.47 8.52 3.90) d
45	C	$-CH_2$-(1,4-phenylene)-$OCH_2CH_2OCH_2Ph$	t_{Bu}	0.30 (SS 11)	70.97 7.72 3.81 (71.13 7.87 3.77)
46	C	$-CH_2$-(1,4-phenylene)-$OCH(CH_3)CH_2OCH_2Ph$ (S)	t_{Bu}	0.37 (SS 12)	
47	C	$-CH_2$-(1,4-phenylene)-CH_2OH	t_{Bu}	0.70 (SS 9)	
48	C	$-CH_2$-(1,4-phenylene)-CH_2OH	$-CH_2CH_2CH_2Ph$	0.25 (SS 9)	

Preparation No.	Method	R^3	R^2	Rf	Analysis % C H N (theoretical in brackets)
49	B		$-CH_2CH_2CH_2Ph$		62.00 6.30 3.69 (62.04 6.30 3.81) b
50	A	$-CH_2OCH_2C(Cl)=CH_2$	$-CH_2CH_2CH_2Ph$	0.30 (SS 9)	59.71 6.78 4.45 (59.78 6.82 4.65) e
51	B	$-CH_2CH_2OCH_2Ph$	$-CH_2CH_2CH_2Ph$	0.35 (SS 9)	64.07 7.09 3.59 (64.42 7.30 3.76) f
52	B	$-CH_2OCH_2Ph$	$-CH_2CH_3$	0.53 (SS 10)	55.28 7.06 5.31 (55.49 6.98 5.39)b
53	B	$-CH_2CH_2O(CH_2)_2CH_3$	$-CH_2CH_3$	0.40 (SS 22)	

54	B	$-CH_2OCH_2C{\equiv}CH$	$-(CH_2)_3Ph$	0.46 (SS 22)	68.28 (68.46	7.27 7.28	5.33 5.32)g
55	B	$-CH_2OCH_2C(=CH_2)CH_3$	$-(CH_2)_3Ph$	0.44 (SS 22)	68.49 (68.39	8.73 8.39	4.95 4.99)e

a. HCl; 0.10 H_2O
b. HCl
c. HCl; 0.25 CH_2Cl_2
d. HCl; 0.20 H_2O
e. 0.20 H_2O
f. HCl; 0.50 H_2O
g. 0.1 H_2O

PREPARATION 56

O-Benzyl-(S)-serine amide

This was obtained in 97% yield as the hydrochloride salt by subjecting N-t-butoxycarbonyl-O-benzyl-(S)-serine amide (Preparation 10) to deprotection Method B; Rf 0.48 (SS 10). Found: C,52.10; H,6.45; N,11.89. $C_{10}H_{14}N_2O_2$; HCl requires C,52.06; H,6.55; N,12.14%.

PREPARATION 57

N-(2,2,2-Trichloroethoxycarbonyl)-aziridine-2(S)-carboxylic acid benzyl ester

a) Trifluoroacetic acid (40 ml) was added dropwise over 10 minutes to a stirred, ice-cooled solution of N-trityl-aziridine-2-carboxylic acid benzyl ester (10 g, 1.0 eq) in methanol/chloroform (1:1, 40 ml) and the reaction mixture stirred for 1.5 hours, when the reaction was complete (by TLC). The reaction mixture was evaporated under vacuum to yield a crystalline residue which was partitioned between diethyl ether (100 ml) and water (50 ml). The aqueous phase was extracted with diethyl ether (2 x) and the organic phases combined. The aqueous phase was neutralised with sodium bicarbonate and reextracted with ether. The combined ether extracts were dried ($MgSO_4$), filtered and evaporated to yield an oil (3.93 g).

b) A solution of the oil from part (a) (3.39 g) in dichloromethane (30 ml) was treated with 2,2,2-trichloroethyl-chloroformate (4.7 g, 1.0 eq) followed by N-methylmorpholine (2.5 g, 1.1 eq). The resulting solution was allowed to warm to room temperature and stirred overnight. The solvent was evaporated under vacuum and the residue dissolved in ethyl acetate (70 ml);

this solution was washed with water (3 x 50 ml), hydrochloric acid (2M, 2 x 50 ml), saturated aqueous sodium bicarbonate solution (2 x 30 ml) and brine (1 x 20 ml), then dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave the crude product as a pale yellow oil, which was chromatographed on silica gel, eluting with mixtures of hexane and ethyl acetate (from 1-10% of ethyl acetate), to yield the pure title compound as an oil (6.8 g, 81%), $[\alpha]_D$ - 20° (c = 0.1, MeOH). Found: C,43.58; H,3.50; N,3.86. $C_{13}H_{12}Cl_3NO_4$ requires C,44.28; H,3.43; N,3.97%.

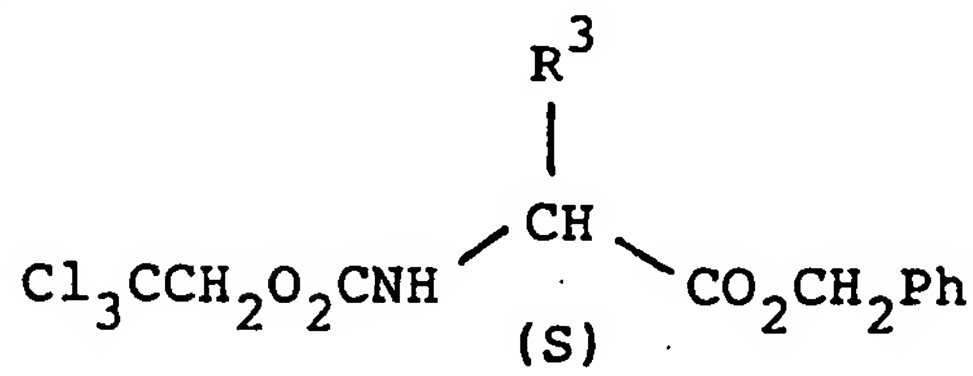
PREPARATION 58

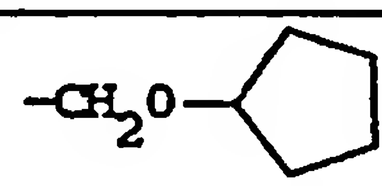
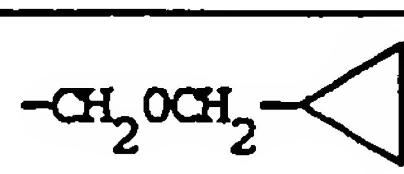
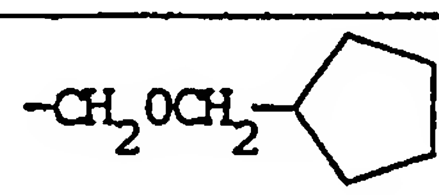
N-(2,2,2-Trichloroethoxycarbonyl)-O-(2-butynyl)-(S)-serine benzyl ester

Boron trifluoride etherate (1 drop) was added to a solution of 2-butyn-1-ol (0.206 g, 2eq) and (S)-N-(2,2,2-trichloroethoxy-carbonyl)-aziridine-2-carboxylic acid benzyl ester (0.52 g, 1.0 eq) in dry dichloromethane (3 ml) and the reaction stirred for 1 hour at room temperature. The solvent was evaporated under vacuum, the residue dissolved in ethyl acetate (20 ml) and the solution washed with water (2 x 10 ml), hydrochloric acid (2M, 2 x 10 ml), saturated aqueous sodium bicarbonate solution (1 x 10 ml) and brine (1 x 10 ml), then dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave an oil which was chromatographed over silica gel, eluting with mixtures of hexane and ethyl acetate (from 1 to 4% of ethyl acetate) to yield the title compound as an oil (48 g, 77%), $[\alpha]_D$ -10° (c = 0.1, MeOH). Rf 0.7 (SS 2). Found: C,48.01; H,4.15; N,3.30. $C_{17}H_{13}Cl_3NO_5$ requires C,48.30; H,4.29; N,3.31%.

PREPARATIONS 59-65

The following compounds were prepared according to the method described above:



Prep. No.	R^3	Rf	Analysis % (Theoretical in brackets) C	H	N
59	$-CH_2O(CH_2)_3CH_3$	0.81 (SS 2)	47.64 (47.85	4.94 5.19	3.02 3.28)
60		0.40 (SS 1)	48.56 (49.28	5.06 5.05	3.10 3.19)
61	$-CH_2OCH(CH_3)_2$	0.44 (SS 1)	46.38 (46.56	4.84 4.88	3.36 3.39)
62	$-CH_2OCHCH_3$ CF_3	0.40 (SS 1)	41.58 (41.18	3.83 3.67	3.06 3.00)
63	$-CH_2OCH_2CF_3$	0.35 (SS 1)	40.44 (39.80	3.51 3.34	3.06 3.10)
64		0.42 (SS 1)	48.20 (48.07	5.23 4.75	3.32 3.29)
65		0.47 (SS 6)	43.05 (43.04	5.49 5.68	3.60 3.59)

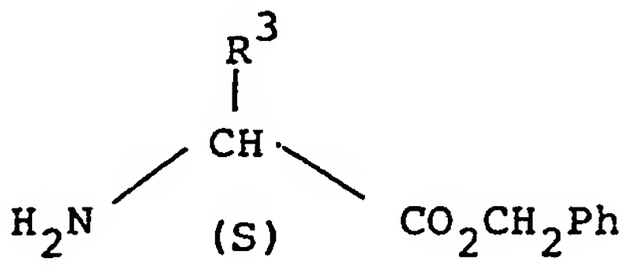
PREPARATION 66 (DEPROTECTION METHOD E)

O-(2-Butynyl)-(S)-serine benzyl ester

Zinc dust (500 mg) was added in one portion to a solution of N-(2,2,2-trichloroethoxycarbonyl)-O-(2-butynyl)-(S)-serine benzyl ester (0.44 g, 1.0 eq) in acetic acid (25 ml) and the mixture stirred at room temperature for 1.5 hours. The zinc was removed by filtration and washed with acetic acid, then the filtrate evaporated under vacuum and the residue azeotroped with toluene. The residue was dissolved in ethyl acetate (20 ml) and the solution washed with saturated aqueous sodium bicarbonate solution (10 ml); the resultant precipitate of sodium acetate was removed by filtration and the filtrate washed again with saturated aqueous sodium bicarbonate solution. The organic phase was extracted with hydrochloric acid (2M, 2 x 100 ml), then the combined extracts overlaid with ethyl acetate and neutralised with solid sodium bicarbonate. The organic layer was separated and the aqueous phase reextracted with ethyl acetate, then the combined organic phases dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate provided the title compound as a yellow oil (200 mg, 78%), Rf 0.71 (SS 19).

PREPARATIONS 67-73

The following compounds were prepared by treatment with zinc in acetic acid according to the method described above:



Preparation No.	R^3	Rf
67	$-CH_2O(CH_2)_3CH_3$	0.62 (SS 19)
68	$-CH_2O$	0.18 (SS 20)
69	$-CH_2OCH(CH_3)_2$	0.18 (SS 20)
70	$-CH_2OCHCH_3$ CF_3	0.17/0.20 (SS 20)
71	$-CH_2OCH_2CF_3$	0.18 (SS 20)
72	$-CH_2OCH_2-$	0.18 (SS 20)
73	$-CH_2OCH_2$	0.31 (SS 22)

PREPARATION 74

4-Methoxymethyl-(S)-phenylalanine t-butyl ester

To a vigorously stirred solution of sodium hydroxide (11.5 g, 0.29 mol) in water (23 ml) were added, sequentially, a solution of (4-methoxymethyl)benzyl bromide (3.1 g, 14.4 mmol) in dichloromethane (30 ml), N-diphenylmethylene glycine t-butyl ester (4.26 g, 14.4 mmol) and N-benzylcinchonidinium chloride (1.21 g, 2.9 mmol). After 2.5 hours the reaction mixture was diluted with dichloromethane (100 ml), then the organic phase separated, washed to neutrality with water, dried ($MgSO_4$) and evaporated under vacuum to provide an oil (7.2 g). Chromatography on silica gel (600 g) using a 4:1 mixture of hexane and ether as eluent afforded a white solid (473 g, 76%) which, on crystallisation from hexane, yielded racemic material (1.71 g), m.p. 81-82°C. Further chromatography of the crystallisation mother liquor on silica gel using the same eluent gave the required (S)-enantiomer as a clear oil (2.44 g, 39%), Rf 0.30 (SS 13), $[\alpha]_D^{25}$ -145° (c = 1.08, CH_2Cl_2). Found: C,77.93; H,7.21; N,3.23. $C_{28}H_{31}NO_3$ requires C,78.29; H,7.27; N,3.26%.

A solution of this product (2.3 g, 5.56 mmol) in ether (50 ml) was vigorously stirred with a mixture of 0.5M hydrochloric acid (12.2 ml) and water (40 ml). After 9 hours the aqueous phase was removed and the ether phase treated two further times in the same way. The combined aqueous phases were basified with 1M aqueous sodium hydroxide solution and extracted with ether. The combined ether extracts were dried ($MgSO_4$) and evaporated under vacuum to give an oil (960 mg) which was chromatographed on silica gel, using ethyl acetate as eluent, to afford the title compound

as a clear oil (860 mg, 59%), Rf 0.35 (SS 8), $[\alpha]_D^{25}$ + 9.7° (c = 1.65, CH_2Cl_2). Found: C,67.73; H,8.54; N,5.10. $C_{15}H_{23}NO_3$ requires C,67.90; H,8.74; N,5.28%.

PREPARATION 75

O-n-Propyl-(S)-serine ethyl ester

a) N-(Triphenylmethyl)-O-(2-propenyl)-(S)-serine ethyl ester (Preparation 23, 1.0 g, 2.4 mmol), dissolved in ethanol (36 ml) and water (4 ml), was hydrogenated over 5% palladium on charcoal (300 mg) at 50 p.s.i. (3.45 bar) and room temperature. After three hours the mixture was filtered through a short arbacel column and the filtrate, on evaporation under vacuum, gave a clear oil (916 mg). Chromatography on silica gel, eluting with a 1:4 mixture of ethyl acetate and hexane gave N-triphenylmethyl-O-n-propyl-(S)-serine ethyl ester (697 mg, 69%). Found: C,78.13; H,8.96; N,3.31. $C_{27}H_{31}NO_3$ requires C,77.66; H,7.48; N,3.35%.

b) The above intermediate (667 mg, 1.6 mmol) was dissolved in a 1.7% solution of concentrated hydrochloric acid in acetone (30 ml); after five hours a further amount (5 ml) of the above acid solution was added to complete the reaction. After a further half hour the mixture was evaporated under vacuum and the residue dried azeotropically with dichloromethane. Trituration with diethyl ether and filtration gave the required product as a white solid (247 mg, 73%), Rf 0.19 (SS 22). Found: C,45.56; H,8.23; N,6.50. $C_8H_{18}ClNO_3$ requires C,45.39; H,8.57; N,6.62%.

PREPARATION 76

3-[2-(R,S)-Tetrahydrofuryl]-(S)-alanine (3-phenyl)propyl ester

a) 3-(2-Furyl)-(S)-alanine (prepared by the method of H. K. Chenault et al, J. Amer. Chem. Soc., 1981, 111, 6354) was reacted with di-t-butyl dicarbonate to give N-t-butoxycarbonyl-3-(2-furyl)-(S)-alanine as a colourless oil, Rf 0.6 (SS 21).

b) The previous product (3.0 g, 0.12 mol) in ethyl acetate (40 ml) was hydrogenated over platinum oxide (200 mg) at 60 p.s.i. (4.1 bar) and room temperature for 2 hours, when uptake was complete. The catalyst was removed by filtration, the filtrate evaporated under vacuum and the residue chromatographed on silica gel eluting with a mixture of dichloromethane, methanol, acetic acid and hexane (90:10:1:150) to give N-t-butoxycarbonyl-3-[(2-(R,S)-tetrahydrofuryl)]-(S)-alanine (2.04 g) as a mixture of two diastereoisomers, Rf 0.20 (SS 26).

c) Alkylation of the previous product with (3-phenyl)propyl bromide following the procedure of Preparation 1, followed by removal of the N-t-butoxycarbonyl protecting group with hydrogen chloride, gave the title compound as a colourless oil, Rf 0.30 (SS 22).

PREPARATION 77

2(S)-trans 1-(4-Ethoxy-2-butenyl)glycine methyl ester hydrochloride

a) n-Butyllithium (3.15 ml, 7.875 mmol, 2.5M in hexane) was added dropwise over 10 minutes under nitrogen to a stirred solution of 2,5-dihydro-3,6-methoxy-2(R)-(2-propyl)pyrazine (1.38 g, 7.49 mmol) in dry tetrahydrofuran, whilst keeping the

temperature below -68°C. After 15 minutes a solution of trans 1-bromo-4-ethoxy-2-butene (1.34 g, 7.49 mmole) in tetrahydrofuran (5 ml) was added over 10 minutes at -78°C. The reaction mixture was then allowed to warm to room temperature overnight, being kept initially at -78°C for at least 5 hours. The solvent was removed under vacuum and the residue partitioned between diethyl ether and water. The organic phase, on drying ($MgSO_4$) and evaporation, gave a golden oil (1.35 g, 65%) which was stirred with 0.25M hydrochloric acid (42.8 ml) for 24 hours. The mixture was evaporated under vacuum and the residue dried azeotropically with dichloromethane followed by toluene.

Di-t-butyl dicarbonate (3.13 g, 14.34 mmol) was added to an ice-cooled solution of the above mixture of esters (2.02 g) and N-methylmorpholine (1.58 ml) in dry dichloromethane (35 ml), and the resulting solution stood at room temperature for 4 days. The solvent was removed under vacuum and the residue partitioned between diethyl ether and water. The organic phase was washed sequentially with water, 1M hydrochloric acid, water, saturated aqueous sodium bicarbonate solution and water, then dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate gave a yellow liquid (1.34 g) which was chromatographed on silica gel; elution with increasing proportions of diethyl ether in hexane gave N-t-butoxycarbonyl-2(S)-trans 1-(4-ethoxy-2-butenyl)glycine methyl ester as an oil (565 mg, 41%), Rf 0.38 (SS 2). Found: C,58.29; H,8.50; N,5.21. $C_{14}H_{25}NO_5$ requires C,58.51; H,8.77; N,4.87%. $[\alpha]_D^{25}$ + 18.6° (c = 1.07, CH_2Cl_2).

b) A stirred, ice-cold solution of the above product (492 mg, 1.7 mmol) in dry diethyl ether (15 ml) was saturated over 1.5 hours with hydrogen chloride gas. After being stirred at 0°C for a further 3 hours, the solution was evaporated under vacuum and the residue dried azeotropically with dichloromethane (x3) to give the title product as a pale yellow foam (320 mg, 84%), Rf 0.32 (SS 14). Found: C, 47.17; H,7.79; N,6.40. $C_9H_{18}ClNO_3$. 0.05 CH_2Cl_2 requires C,47.68; H,7.56; N,6.14%.

PREPARATION 78

2(S)-trans 1-(4-Methoxy-2-butenyl)glycine methyl ester hydrochloride

This was similarly prepared following the procedures described above and was obtained as a white powder Rf 0.30 (SS 22). Found: C,45.17; H,7.54; N,6.71. $C_8H_{16}NClO_3$; 0.2 H_2O requires C,45.05; H,7.75; N,6.57%.

PREPARATION 79

2(S)-(4-Methoxy-1-butyl)glycine methyl ester

This compound was obtained from the previous product (free base), according to Preparation 75a, as a yellow oil, Rf 0.15 (SS 22).

PREPARATION 80

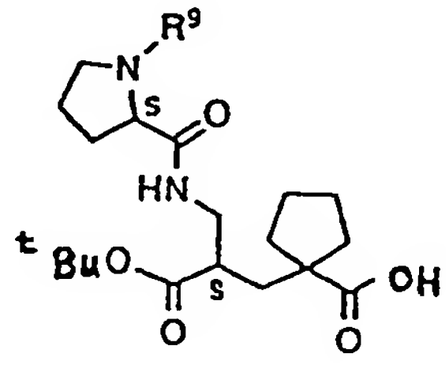
1-{3-[N-t-Butoxycarbonyl-(S)-prolylamino]-2(S)-t-butoxycarbonyl-propyl}cyclopentane carboxylic acid

N-t-Butoxycarbonyl-(S)-proline 4-nitrophenyl ester (2.89 g,

8.59 mmol) was added to a stirred suspension of 1-[3-amino-2(S)-t-butoxycarbonylpropyl]cyclopentane carboxylic acid sodium salt (EP-A-358398; 1.70 g, 5.90 mmol) in dry dichloromethane (30 ml). After 24 hours the reaction mixture was evaporated under vacuum and the residue allowed to stand for a further 24 hours before being partitioned between ethyl acetate (200 ml) and 2M hydrochloric acid (100 ml). The organic phase was separated, washed successively with 2M hydrochloric acid (2 x 50 ml), saturated aqueous sodium bicarbonate solution (4 x 50 ml), more 2M hydrochloric acid (50 ml) and saturated brine (50 ml), dried ($MgSO_4$), filtered, and the filtrate evaporated under vacuum. The resulting yellow oil (4.59 g) was purified by chromatography on silica gel (200 g), using an elution gradient of 1% acetic acid in ethyl acetate / 0 to 10% methanol, to furnish the title compound as a cream foam (1.096 g, 40%), Rf 0.53 (SS 14), $[\alpha]_D^{25}$ -161° (c = 0.1, MeOH). Found: C,61.20; H,8.81; N,5.43. $C_{24}H_{40}N_2O_7$ requires C,61.51; H,8.60; N,5.98%.

PREPARATIONS 81-82

The following compounds of formula (V) were prepared from the appropriate proline derivatives using the procedure described above for Preparation 80.



Preparation No.	R^9	Rf	Analysis % (Theoretical in brackets) C	H	N
81	$PhCH_2CO_2-$	0.25 (SS 8)	64.75 (64.52	7.60 7.62	5.53 5.57)
82	$(Cl)_3CCH_2CO_2-$	0.50 (SS 21)	48.74 (48.58	5.85 6.12	5.69 5.15)

PREPARATION 83

1-[3-Benzyloxycarbonylamino-2(S)-t-butoxycarbonylpropyl]cyclopentane carboxylic acid

To a stirred, ice-cold suspension of 1-[3-amino-2(S)-t-butoxycarbonylpropyl]cyclopentane carboxylic acid sodium salt (1.0 g, 3.4 mmol) in dry dichloromethane (15 ml) were added, sequentially, N-methylmorpholine (0.4 ml, 4.0 mmol) and N-benzyloxycarbonyloxysuccinimide (930 mg, 3.75 mmol). After 1 hour the ice bath was removed and stirring was continued for 24 hours. The reaction mixture was evaporated under vacuum, then the residue partitioned between ethyl acetate and water. The organic phase was separated, washed with 1M hydrochloric acid and water, dried ($MgSO_4$) and filtered. Evaporation under vacuum of the filtrate provided an oil (1.4 g) which was purified by chromatography on silica gel, using a 7:3 mixture of ether and hexane as eluent, to give the title compound as a gum (900 mg), Rf 0.50 (SS 2).

Biological activity

The following Table illustrates the dual in vitro enzyme inhibitory activities for a range of the compounds of the invention.

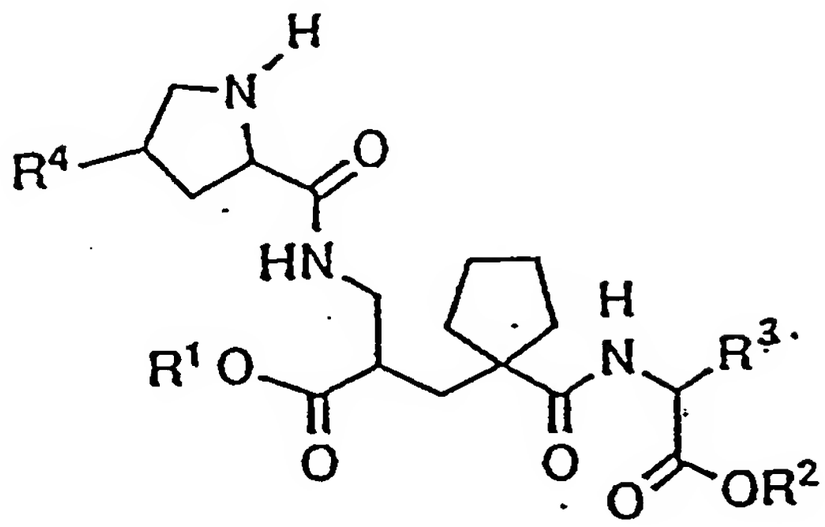
	IC_{50} (M)	
EXAMPLE NUMBER	ANGIOTENSIN CONVERTING ENZYME (ACE)	NEUTRAL METALLOENDOPEPTIDASE (E.C. 3.4.24.11)
86	1.3×10^{-8}	3.0×10^{-8}
87	1.4×10^{-8}	2.0×10^{-8}
92	2.6×10^{-8}	2.0×10^{-8}
128	1.6×10^{-9}	4.1×10^{-8}
130	4.0×10^{-8}	1.8×10^{-8}
131	3.7×10^{-8}	4.8×10^{-8}
133	3.2×10^{-8}	4.0×10^{-8}
134	1.6×10^{-8}	3.4×10^{-8}
136	1.5×10^{-8}	1.5×10^{-8}
137	1.4×10^{-8}	3.6×10^{-8}
138	2.4×10^{-9}	3.2×10^{-8}
144	6.6×10^{-8}	3.3×10^{-8}
145	1.1×10^{-8}	4.1×10^{-8}

Safety profile

The prodrugs of the invention have been tested orally in rat at doses up to 10 mg/Kg and the diacids of the invention have been tested intravenously in rat at doses up to 10 mg/Kg. No signs of adverse acute toxicity were observed.

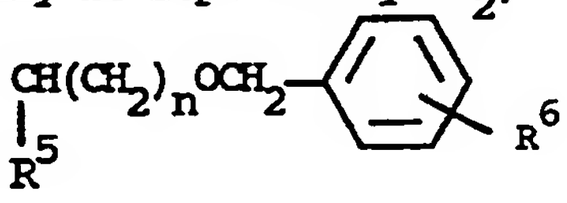
CLAIMS

1. A compound of formula:



(I)

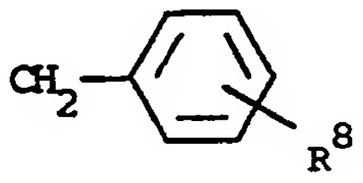
wherein R^1 and R^2 are each independently H or a biolabile ester-forming group, and either or both of OR^1 and OR^2 may optionally be replaced by NH_2;

R^3 is (a) 

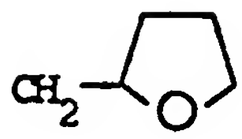
wherein R^5 is H or methyl, R^6 is H or halo, and n is 0 or 1;

(b) CH_2OR^7

wherein R^7 is C_1-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_7 cycloalkyl, (C_1-C_4 alkoxy)C_1-C_6 alkyl, (C_1-C_4 alkoxy)C_3-C_6 alkenyl, (halo)C_3-C_6 alkenyl, (C_3-C_7 cycloalkyl)C_1-C_6 alkyl or (CF_3)C_1-C_6 alkyl;

(c) 

wherein R^8 is CH_2OH, CH_2OCH_3, $OCH(R^5)CH_2OH$ or $OCH_2CH_2OCH_3$ and R^5 is as previously defined;

(d)  or

(e) (C_1-C_4 alkoxy)C_3-C_6 alkenyl or (C_1-C_4 alkoxy)C_2-C_6 alkyl;

and R^4 is H or hydroxy;

and pharmaceutically acceptable salts thereof.

2. A compound as claimed in claim 1 wherein R^1 and R^2 are each independently selected from H, C_1-C_5 alkyl, C_5-C_7 cycloalkyl, (cyclohexyl)C_1-C_3 alkyl, (phenyl)C_1-C_3 alkyl, 1-(C_2-C_5 alkanoyloxy)C_1-C_4 alkyl, 1-(C_5-C_6 cycloalkylacetoxy)C_1-C_4 alkyl, 1-(C_5-C_7 cycloalkylcarboxy)C_1-C_4 alkyl, 1-(2-indanylcarboxy)C_1-C_4 alkyl, 1-(benzoyloxy)C_1-C_4 alkyl, 3-phthalidyl, 1-(C_1-C_4 alkoxy-carbonyloxy)C_1-C_4 alkyl, [4-(5-[C_1-C_4 alkyl]-1,3-dioxolen-2-onyl)]methyl, acetonyl, indanyl and pyridyl.

3. A compound as claimed in claim 2 wherein R^1 and R^2 are each independently selected from H, methyl, ethyl, (3-cyclohexyl)-propyl, (3-phenyl)propyl, pivaloyloxymethyl, 1-(cyclohexyl-acetoxy)ethyl, 1-(cyclohexylcarboxy)ethyl, 1-(2-indanylcarboxy)-ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl or [4-(5-methyl-1,3-dioxolen-2-onyl)]methyl.

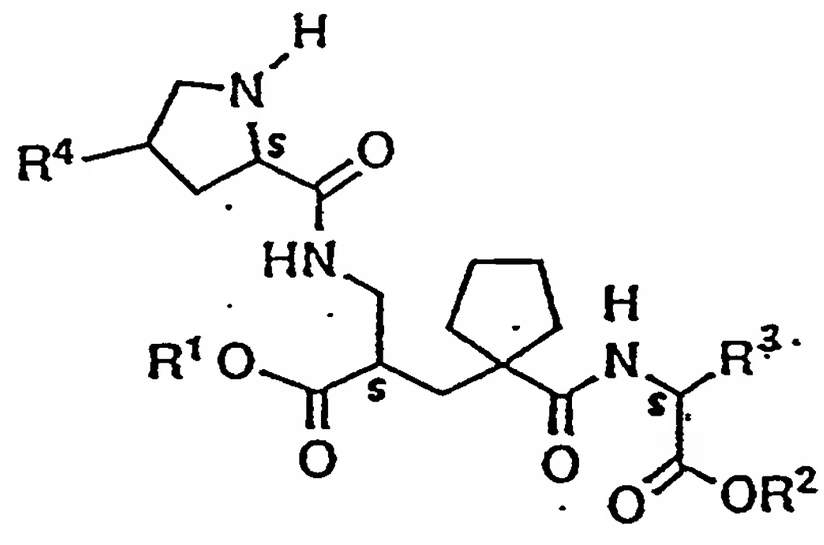
4. A compound as claimed in claim 3 wherein R^3 is benzyloxy-methyl, 1-(2-butenyl)oxymethyl, 1-(4-methoxy-2-butenyl)oxymethyl or 2-chloro-2-propenyloxymethyl, and R^4 is H.

5. A compound as claimed in claim 4 wherein R^1 is H, and R^2 is methyl, (3-phenyl)propyl or (3-cyclohexyl)propyl.

6. A compound as claimed in claim 4 wherein R^1 is pivaloyloxymethyl, 1-(cyclohexylacetoxy)ethyl, 1-(cyclohexylcarboxy)ethyl, 1-(2-indanylcarboxy)ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl or [4-(5-methyl-1,3-dioxolen-2-onyl)]methyl, and R^2 is ethyl.

7. A compound as claimed in claim 1 wherein both R^1 and R^2 are H.

8. A compound as claimed in claims 1 to 7 wherein the preferred stereoisomer is of formula:

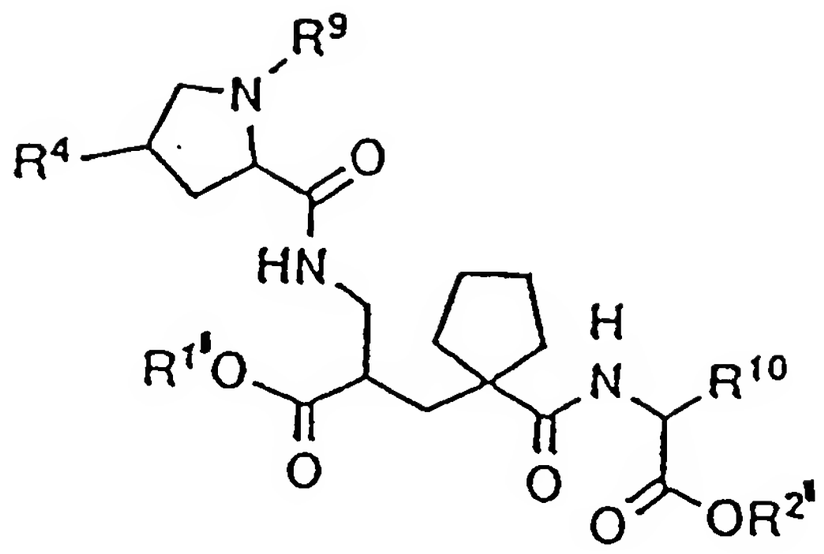


9. A pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

10. A compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either, for use in medicine.

11. The use of a compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either, for the manufacture of a medicament for the treatment of hypertension, heart failure or renal insufficiency.

12. A compound of formula:



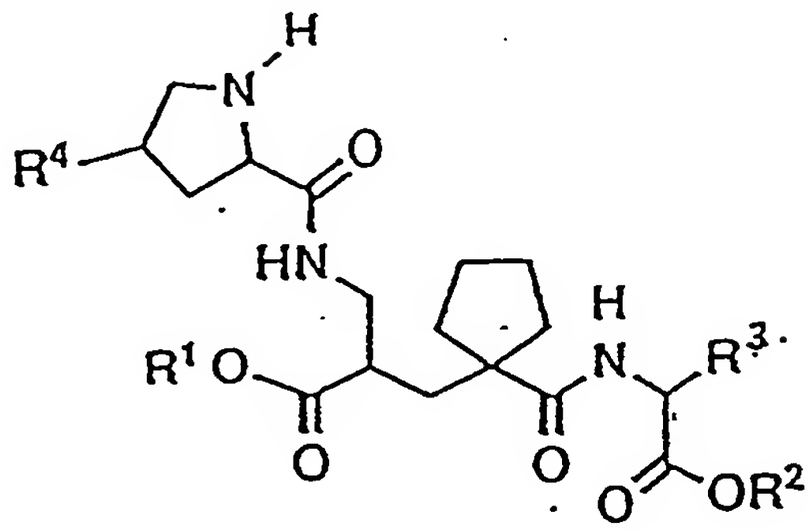
(IV)

wherein $R^{1'}$ and $R^{2'}$ are as defined in claim 1 for R^1 and R^2 respectively but are not H, and $OR^{1'}$ and $OR^{2'}$ are as defined in claim 1 for OR^1 and OR^2, R^4 is as defined in claim 1, R^9 is a conventional amino acid N-protecting group and R^{10} is as defined in claim 1 for R^3 with any reactive groups therein optionally protected.

13. A compound as claimed in claim 12 wherein $R^{1'}$ and $R^{2'}$ are each independently selected from t-butyl and benzyl.

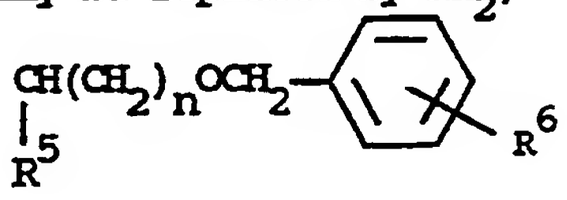
14. A method for the prophylactic or curative treatment of hypertension, heart failure or renal insufficiency in a human being, which comprises administering to said human being an effective amount of a compound of formula (I), a pharmaceutically acceptable salt thereof, or a pharmaceutical composition containing either.

15. A process for the preparation of a compound of formula:



(I)

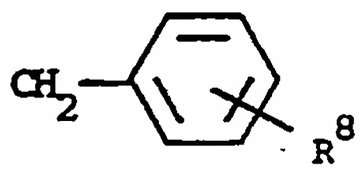
wherein R^1 and R^2 are each independently H or a biolabile ester-forming group, and either or both of OR^1 and OR^2 may optionally be replaced by NH_2;

R^3 is (a) 

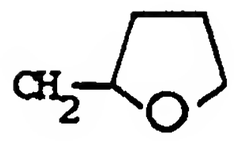
wherein R^5 is H or methyl, R^6 is H or halo, and n is 0 or 1;

(b) CH_2OR^7

wherein R^7 is C_1-C_6 alkyl, C_3-C_6 alkenyl, C_3-C_6 alkynyl, C_3-C_7 cycloalkyl, (C_1-C_4 alkoxy)C_1-C_6 alkyl, (C_1-C_4 alkoxy)C_3-C_6 alkenyl, (halo)C_3-C_6 alkenyl, (C_3-C_7 cycloalkyl)C_1-C_6 alkyl or (CF_3)C_1-C_6 alkyl;

(c) 

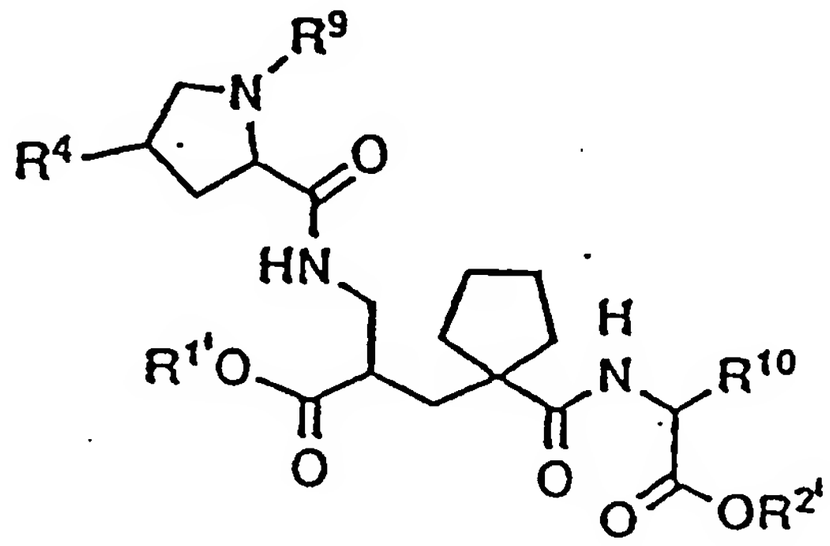
wherein R^8 is CH_2OH, CH_2OCH_3, $OCH(R^5)CH_2OH$ or $OCH_2CH_2OCH_3$ and R^5 is as previously defined;

(d)  or

(e) (C_1-C_4 alkoxy)C_3-C_6 alkenyl or (C_1-C_4 alkoxy)C_2-C_6 alkyl;

and R^4 is H or hydroxy;

or a pharmaceutically acceptable salt thereof, which comprises removing R^9, any protecting group present in R^{10}, and optionally either one or both of any biolabile ester-forming groups $R^{1'}$ and $R^{2'}$ which may be present, from a compound of formula:



(IV)

wherein R^4 is as previously defined, R^9 is a conventional amino acid N-protecting group, R^{10} is as defined for R^3 with any reactive groups therein optionally protected, and $R^{1'}$ and $R^{2'}$ are as defined for R^1 and R^2 but are not H, and $OR^{1'}$ and $OR^{2'}$ are as defined for OR^1 and OR^2, and optionally isolating as, or forming, a pharmaceutically acceptable salt of the product.

16. A process as claimed in claim 15 wherein R^1 and R^2 are each independently selected from H, C_1-C_5 alkyl, C_5-C_7 cycloalkyl,

(cyclohexyl)C_1-C_3 alkyl, (phenyl)C_1-C_3 alkyl, 1-(C_2-C_5 alkanoyloxy)C_1-C_4 alkyl, 1-(C_5-C_6 cycloalkylacetoxy)C_1-C_4 alkyl, 1-(C_5-C_7 cycloalkylcarboxy)C_1-C_4 alkyl, 1-(2-indanylcarboxy)C_1-C_4 alkyl, 1-(benzoyloxy)C_1-C_4 alkyl, 3-phthalidyl, 1-(C_1-C_4 alkoxy-carbonyloxy)C_1-C_4 alkyl, [4-(5-[C_1-C_4 alkyl]-1,3-dioxolen-2-onyl)]methyl, acetonyl, indanyl and pyridyl.

17. A process as claimed in claim 16 wherein R^1 and R^2 are each independently selected from H, methyl, ethyl, (3-cyclohexyl)-propyl, (3-phenyl)propyl, pivaloyloxymethyl, 1-(cyclohexyl-acetoxy)ethyl, 1-(cyclohexylcarboxy)ethyl, 1-(2-indanylcarboxy)-ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl or [4-(5-methyl-1,3-dioxolen-2-onyl)]methyl.

18. A process as claimed in claim 17 wherein R^1 is H, and R^2 is methyl, (3-phenyl)propyl or (3-cyclohexyl)propyl.

19. A process as claimed in claim 17 wherein R^1 is pivaloyloxymethyl, 1-(cyclohexylacetoxy)ethyl, 1-(cyclohexyl-carboxy)ethyl, 1-(2-indanylcarboxy)ethyl, 1-(benzoyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl or [4-(5-methyl-1,3-dioxolen-2-onyl)]-methyl, and R^2 is ethyl.

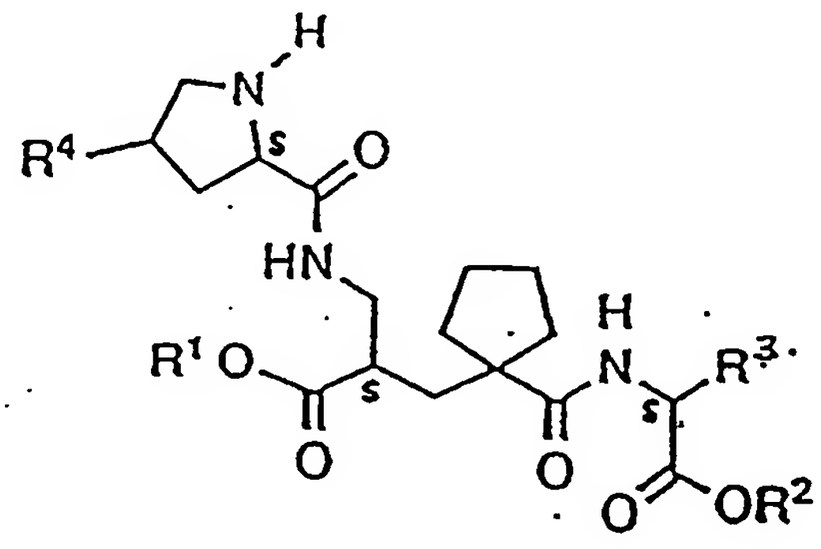
20. A process as claimed in any one of claims 15 to 19 wherein R^9 is t-butoxycarbonyl, benzyloxycarbonyl or 2,2,2-trichloro-ethoxycarbonyl and is removed by acidolysis, hydrogenolysis and treatment with zinc in glacial acetic acid respectively.

21. A process for the preparation of a compound of formula (I) wherein both R^1 and R^2 are H and R^3 and R^4 are as defined in claim 15, or a pharmaceutically acceptable salt thereof, which comprises subjecting a compound of formula (I) wherein R^1 is H, R^2 is a biolabile ester-forming group and R^3 and R^4 are as defined in

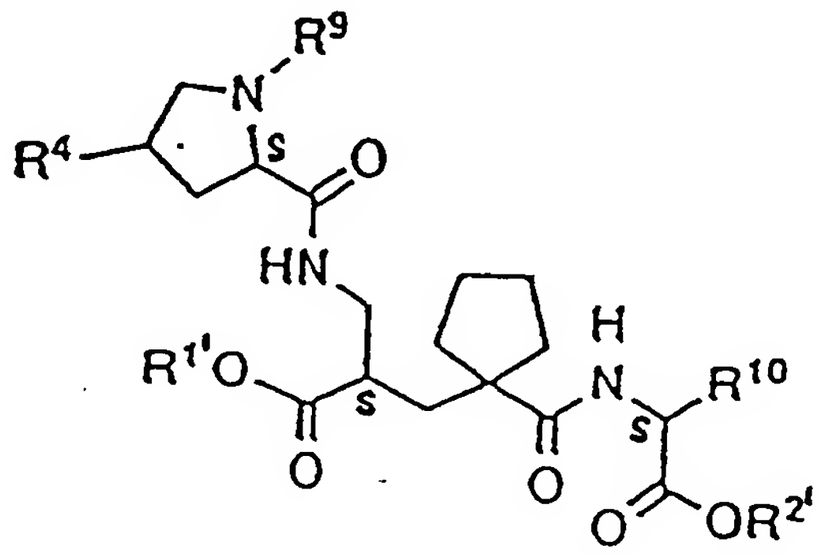
claim 15 to base hydrolysis and optionally isolating as, or forming, a pharmaceutically acceptable salt of the product.

22. A process as claimed in any one of claims 15 to 21 wherein R^3 is benzyloxymethyl, 1-(2-butenyl)oxymethyl, 1-(4-methoxy-2-butenyl)oxymethyl or 2-chloro-2-propenyloxymethyl, and R^4 is H.

23. A process as claimed in any one of claims 15 to 22 wherein the preferred stereoisomer of the product is of formula:



and that of the precursor intermediate is of formula:



INTERNATIONAL SEARCH REPORT

International Applicat. . No PCT/EP 92/00321

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 D 207/16 A 61 K 31/40 C 07 D 405/12
C 07 D 401/12

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 D 207/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,0358398 (PFIZER) 14 March 1990, see claim 1 (cited in the application) ---	1-23
A	Journal of Medicinal Chemistry, vol. 15, no. 8, 1972, M.C. KHOSLA et al.: "Synthesis of some analogs of angiotensin II as specific antagonists of the parent hormone", pages 792-795, see entire publication ---	1-23
A	Journal of Medicinal Chemistry, vol. 13, no. 2. March 1970, N.C. CHATURVEDI et al.: "Analogs of angiotensin II. I. Solid phase synthesis", pages 177-181, see entire publication ---	1-23
A	EP,A,0274234 (PFIZER) 13 July 1988, see entire document -----	1-23

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26-03-1992	27 APR 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mme N. KUIPER

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 14 is directed to a method of treatment of (diagnostic method practised on) the human/ animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9200321 SA 56109

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/04/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0358398	14-03-90	AU-B-	604195	06-12-90
		AU-A-	4105289	08-03-90
		JP-A-	2124862	14-05-90
EP-A- 0274234	13-07-88	AU-B-	595082	22-03-90
		AU-A-	8240787	07-07-88
		DE-A-	3772950	17-10-91
		JP-A-	63165353	08-07-88
		SU-A-	1612996	07-12-90
		US-A-	5030654	09-07-91